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THYROID HORMONE RESPONSE TO VARYING ENERGY AVAILABILITIES IN TRAINED WOMEN

BY

SHERI L. FOSTER ©

A thesis submitted to the Faculty of Graduate Studies and Research in partial fulfillment of the requirements for the degree of Master of Science

FACULTY OF PHYSICAL EDUCATION AND RECREATION

Edmonton, Alberta

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Life is a Journey... Not a Destination

- Anonymous

ABSTRACT

The effects of varying energy availabilities (dietary energy intake minus energy expended during exercise) on thyroid metabolism was examined in 29 healthy, young, physically trained eumenorrheic (EUM) women. Subjects were quasi-randomly assigned to 5 groups of energy availability of 5.5, 11.4, 18.1, 25.0 and 37.4 kcal·kg FFM^{-1}·day^{-1}. The 7-day study began on the second through seventh day of each subject's menstrual cycle and involved 7-consecutive days of obtaining blood and urine samples and body weight between the hours of 5:30 A.M. and 10:30 A.M.. On days 3, 4, 5 and 6 of the experiment (ie. treatment days), each participant completed a supervised fixed volume of exercise equivalent to 30.0 kcal·kg FFM^{-1}·day^{-1} in a laboratory on a Monark cycle ergometer. This exercise was performed at approximately 90% of the subject's ventilatory threshold (VT) in 30 minute bouts with 10 minutes of rest. In addition to the 4 days of exercise, subjects consumed a liquid nutritional supplement as their only food source (Ensure, Ross Laboratories, Columbus, Ohio) which was supplied by lab personnel. The energy provided by this drink established each energy availability group. Baseline concentrations for thyroxine (T_4), triiodothyronine (T_3), free T_4 (fT_4), free T_3 (fT_3), and reverse T_3 (rT_3) were determined by averaging hormone concentrations prior to treatment (ie. days 1, 2 and 3). An estimate of each subject's response to treatment was determined by subtracting the subject's thyroid hormone concentrations on experiment day 7 (ie.

This thesis is dedicated to
all athletic women
who
push the limits,
break barriers and
go after their dreams.

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LIST OF ABBREVIATIONS

AMEN - amenorrheic/amenorrhea
BMR - basal metabolic rate
CHO - carbohydrate
CV - coefficient of variation
E_2 - estradiol
EAT - eating attitudes test
EUM - eumenorrheic/eumenorrhea
FFM - fat free mass
FM - fat mass
FSH - follicle stimulating hormone
fT_3 - free T_3
fT_4 - free T_4
GH - growth hormone
GnRH - gonadotropin-releasing hormone
HR - heart rate
HR_{max} - maximum heart rate
HPA - hypothalamic-pituitary-adrenal
HPT - hypothalamic-pituitary-thyroid
IGF-I - insulin-like growth factor-I
IGFBP-I - insulin-like growth factor binding protein-I
kcal - kilocalorie
$kcal \cdot day^{-1}$ - kcal per day
$kcal \cdot kg\ body\ wt^{-1} \cdot day^{-1}$ - kcal per kilogram of body weight per day
$kcal \cdot kg\ FFM^{-1} \cdot day^{-1}$ - kcal per kilogram of fat free mass per day
LBM - lean body mass
LH - luteinizing hormone
LSD - least significant difference
OLIGO - oligomenorrheic/oligomenorrhea
RER - respiratory exchange ratio
RIA - radioimmunoassay
RMR - resting metabolic rate
RPM - revolutions per minute
rT_3 - reverse rT_3 (3,5',3'-triiodothyronine)
T_0 - thyronine
T_1 - monoiodothyronine
T_2 - diiodothyronine
T_3 - triiodothyronine (3,5,3'-triiodothyronine)
T_4 - thyroxine (3,5,3',5'-tetraiodothyronine)
RQ - respiratory quotient
TBG - thyroxine-binding globulin
TRH - thyrotropin-releasing hormone
TSH - thyroid-stimulating hormone
TTR - transthyretin
$VO_{2\,max}$ - maximum oxygen consumption
VT - ventilatory threshold

CHAPTER I

INTRODUCTION

PURPOSE

Reproductive dysfunction in physically trained women may be an outcome of insufficient caloric compensation for their enormous energy expenditures. In the absence of adequate available energy (defined as dietary energy intake minus energy expended during exercise), the energetic demands of the menstrual cycle may not be met and result in a variety of menstrual disturbances (eg. luteal phase deficiency, anovulation, amenorrhea) (Loucks, 1996). A decrease in 3,5,3'-triiodothyronine (T_3) is an indicator of energy deficiency, and has been found in physically trained amenorrheic (AMEN) women (Baer, 1993; Harber et al., 1998; Loucks et al., 1992; Marcus et al., 1985; Myerson et al., 1991). Similarly, an increase in reverse-T_3 (rT_3) occurs during the acute stages of very low caloric intakes or fasting, and therefore also appears to be an indicator of energy deficiency (Azizi, 1978; LoPresti et al., 1991; Palmblad et al., 1977; Serog et al., 1982; Vagenakis et al., 1975). Loucks & Heath (1994a) established energy availability thresholds in sedentary eumenorrheic (EUM) women, in which T_3 levels declined and rT_3 levels increased. Similar data are not available for the athletic AMEN and EUM woman. Since a physically trained woman's caloric intake is often identical to her sedentary counterpart (Beidleman et al., 1995; Broocks et al., 1990; Dahlstrom et al., 1995; Deuster et al., 1986; Drinkwater et al., 1984; Edwards et al., 1993; Harber

et al., 1998; Loucks et al., 1992; Mulligan & Butterfield, 1990; Myerson et al., 1991; Perry et al., 1996; Schwartz et al., 1981; Schweiger et al., 1988; Snead et al., 1992; Watkin et al., 1991; Wilmore et al., 1992), it has been hypothesized that these trained women are more energy efficient (Baer, 1993; Kaiserauer et al. 1989; Marcus et al. 1985; Mulligan & Butterfield, 1990; Myerson et al., 1991; Nelson et al., 1986; Perry et al. 1996). This efficiency might be expressed as different energy availability thresholds compared to sedentary women. It was therefore the purpose of this research project to examine the relationship between thyroid metabolism and energy availability in healthy, young, physically trained, EUM women.

JUSTIFICATION FOR THE STUDY

Physically trained women participating in sports which emphasize a lean physique or a low body weight are more likely to adopt weight loss strategies in hope of attaining the "ideal" or "optimal" weight. Such sports disciplines include sports in which performance is subjectively scored (e.g. dance, figure skating and gymnastics), endurance sports favouring participants with a low body weight (e.g. distance running, cycling and cross-country skiing), sports in which body contour-revealing clothing is worn for competition (e.g. volleyball, swimming, diving and running), sports using weight categories for participation (e.g. horse racing, martial arts and rowing), and sports in which prepubertal body habits favours success (e.g. figure skating, gymnastics and diving) (Nattiv et al., 1994; West, 1998).

Research suggests that the magnitude of change in energy availability may be detrimental to the woman (Loucks & Callister, 1993; Loucks & Heath, 1994a). For example, energy deficient physically active

women are at an increased risk for nutritional deficiencies (Deuster et al., 1986), cardiovascular disease (Friday et al., 1993), stress fractures, premature osteoporosis (Drinkwater, 1989; Nelson et al., 1986), the development of eating disorders (Sundgot-Borgen, 1993; Sundgot-Borgen, 1994), slower recovery times following intense exercise (Harber et al., 1998) and reductions in metabolic rate (Myerson et al., 1991), which may put these women at risk for increased fat mass once training is reduced or terminated (Baer, 1993).

Moreover, some researchers believe that inadequate nutrition plays a role in reproductive dysfunction (De Souza et al., 1998; Loucks et al., 1994b; Loucks et al., 1994c; Loucks et al., 1995; Williams et al., 1995; Loucks & Heath, 1998a; Loucks et al., 1998b), as physically trained AMEN women have reported consuming significantly fewer calories then their EUM counterparts (Baer, 1993; Kaiserauer et al., 1989; Marcus et al., 1985; Nelson et al., 1986). This hypothesis is strengthened with findings of low T_3 levels in physically trained AMEN women (Baer, 1993; Harber et al., 1998; Loucks et al., 1992; Marcus et al., 1985; Myerson et al., 1991). Moreover, one would expect increased levels of rT_3 in AMEN athletes, as an increase in rT_3 is also a sign of energy deficiency, in the acute stages of low caloric or fasting studies (Azizi, 1978; LoPresti et al., 1991; Palmblad et al., 1977; Serog et al., 1982; Vagenakis et al., 1975). However, Loucks et al. (1992) found rT_3 levels lower compared to EUM athletes, whereas no differences were found by Myerson et al. (1991) and Wilmore et al. (1992). Identification of the levels of energy availability at which a reduction in T_3 and an increase in rT_3 occurs, has been identified in sedentary EUM women (Loucks & Heath, 1994a). However, it is hypothesized that these thresholds may be different in the physically active

woman, as numerous cross-sectional studies have found that weight stable athletic women report energy intakes similar to those of sedentary EUM women (Beidleman et al., 1995; Broocks et al., 1990; Dahlstrom et al., 1995; Deuster et al., 1986; Drinkwater et al., 1984; Edwards et al., 1993; Harber et al., 1998; Loucks et al., 1992; Mulligan & Butterfield, 1990; Myerson et al., 1991; Perry et al., 1996; Schwartz et al., 1981; Schweiger et al., 1988; Snead et al., 1992; Watkin et al., 1991; Wilmore et al., 1992).

This research project implemented a modified experimental design from Loucks & Heath's (1994a), to act as a comparative template in an attempt to identify if energy availability thresholds exist in physically trained EUM women. If a level of energy availability is identified at which a reduction in T_3 and/or an increase in rT_3 occurs, this may assist in developing nutritional strategies or weight control programs for EUM athletic women, whereby these hormone levels can be maintained, in hope that metabolic rate and perhaps reproductive function will not be compromised.

HYPOTHESIS

It was hypothesized that physically trained EUM women would have thyroid thresholds at energy availabilities between 11.0 and 19.0 kcal·kg FFM^{-1}·day^{-1} for T_3 and fT_3, and for rT_3 and fT_4 between 5.0 and 11.0 kcal·kg FFM^{-1}·day^{-1}.

DELIMITATIONS

This study examined 29 healthy, young, physically active, non smoking, women. Admittance into the study required that the volunteers be: i) 17 to 35 years of age; ii) have a documented mean menstrual cycle length for the previous 3 menstrual cycles of 24 to 37 days; iii) free from use of oral contraceptives (for at least the previous 3 months) or any other medication known to effect hormonal status; iv) no reported history of heart disease, liver disease, renal disease, diabetes or thyroid disorders; and v) have a $\dot{V}O_{2max} \geq 44.8$ ml·kg^{-1}·min^{-1}.

LIMITATIONS

1. Random Selection

The subjects who participated in this study were volunteers from the University of Alberta and surrounding community. Any woman who met the entry screening criteria was eligible to partake in the study. Subjects were quasi-randomly assigned to various energy availability groups and therefore true random assignment was not met (refer to 'Group Assignment' in Chapter 3: Methods and Procedures).

2. Sample Size

A power calculation based on Loucks & Heath's (1994a) T_3 threshold was completed to determine the number of subjects needed for each energy availability group. This calculation indicated that 18 subjects were needed per group (using an alpha of 5% and a beta of 80%) (Florey, 1993). Due to the

amount of time and financial restrictions involved with the study, and the difficulty of finding subjects to meet all of the entry criteria, this sample size was not met. Each energy availability group resulted in having 6 subjects, with 1 group having only 5 women. In contrast, Loucks & Heath (1994a) had 4 groups of energy availability ($n = 6, 8, 8, 5$ in ascending energy availability group order). By implementing a small sample size in the present study, the power of finding significant differences between energy availability groups was decreased.

3. Menstrual Status

An entry screening questionnaire was implemented to document menstrual status with subjects having to record day 1 (onset of menstrual flow) of their menstrual cycles over 3 consecutive months. Subclinical alterations of the menstrual cycle (eg. luteal suppression) were not examined. Consequently, some or all subjects may have had some degree of menstrual dysfunction and thus their metabolic status (Myerson et al., 1991) may have been different from others with no alterations in their menstrual cycle. Therefore, these women (with subclinical menstrual cycle alterations) may have responded differently to the treatment.

4. Habitual Dietary Intake

Each subject's habitual dietary intake was determined through the implementation of a 7-day dietary record. This analysis was completed permitting a comparison between their habitual diet and the

diet that they consumed while participating in the study. Nonetheless, there are inherent problems associated with using dietary records as under-reporting (Beidleman et al. 1995; Dahlstrom et al., 1995; Edwards et al., 1993; Nelson et al., 1986; Snead et al., 1992; Wilmore et al., 1992), under-eating (Dahlstrom et al., 1995; Wilmore et al., 1992) and under-estimating (Dahlstrom et al. 1995; Edwards et al., 1993) may occur.

DEFINITIONS OF TERMS

I. Expressing Hormonal Concentrations (Table I.I)

Metric units have been used throughout this thesis to express various hormonal concentrations. The table below provides the conversion factors necessary to convert the metric units into Systeme International (SI) Units.

Table I.I ***Hormone Conversion Factors***

Hormone	Metric Unit	Conversion Factor	SI Unit Symbol
E_2	$pg \cdot ml^{-1}$	3.671	$pmol \cdot L^{-1}$
T_4	$\mu g \cdot dL^{-1}$	12.87	$nmol \cdot L^{-1}$
T_3	$ng \cdot dL^{-1}$	0.01536	$nmol \cdot L^{-1}$
fT_4	$ng \cdot dL^{-1}$	12.87	$pmol \cdot L^{-1}$
fT_3	$pg \cdot ml^{-1}$	1.536	$pmol \cdot L^{-1}$
rT_3	$ng \cdot ml^{-1}$	1.54	$nmol \cdot L^{-1}$

2. The Human Menstrual Cycle (Figure I.I)

The menstrual cycle involves a hormonal-feedback system incorporating hypothalamic and anterior pituitary hormones from the brain, and ovarian hormones. The cycle is regulated by the hypothalamus, which produces and secretes gonadotrophin releasing hormone (GnRH), which stimulates the anterior pituitary gland to release luteinizing hormone (LH) and follicle stimulating hormone (FSH) (Golub, 1992). The ovaries have a dual function: maturation of germ cells (ie. oogenesis) and steroidogenesis. Each germ cell is enclosed within a larger structure, termed the follicle. After extrusion of the ovum during ovulation, the follicle is transformed into a new endocrine structure, the corpus luteum. FSH is primarily involved in stimulating the growth of ovarian follicles, while LH controls ovulation and regulates steroidogenesis. The woman has 2 major sex steroids: estradiol (E_2) and progesterone (Rhoades & Tanner, 1995).

A normal menstrual cycle varies between 23 to 35 days, with an average length of 28 days (Otis, 1992). Cycle length is calculated from the first day of menstrual onset (Day 1) until the beginning of the next menses. The menstrual cycle consists of an ovarian cycle which is divided into a follicular and luteal phase, with ovulation marking the midpoint. The interval from ovulation to the onset of menstruation (the luteal phase) is relatively constant, averaging 14 ± 2 days, and is dictated by the fixed life span of the corpus luteum. In contrast, the interval from the onset of menses to ovulation (the follicular phase) is more

variable and accounts for the differences in cycle lengths among ovulating women (Otis, 1992). For the purpose of explaining a hypothetical 28 -day menstrual cycle, the cycle will be divided as follows: menses (days 0-5), follicular phase (days 0-13), LH surge and ovulation (days 13-14) and the luteal phase (days 14-28) (Rhoades & Tanner, 1995).

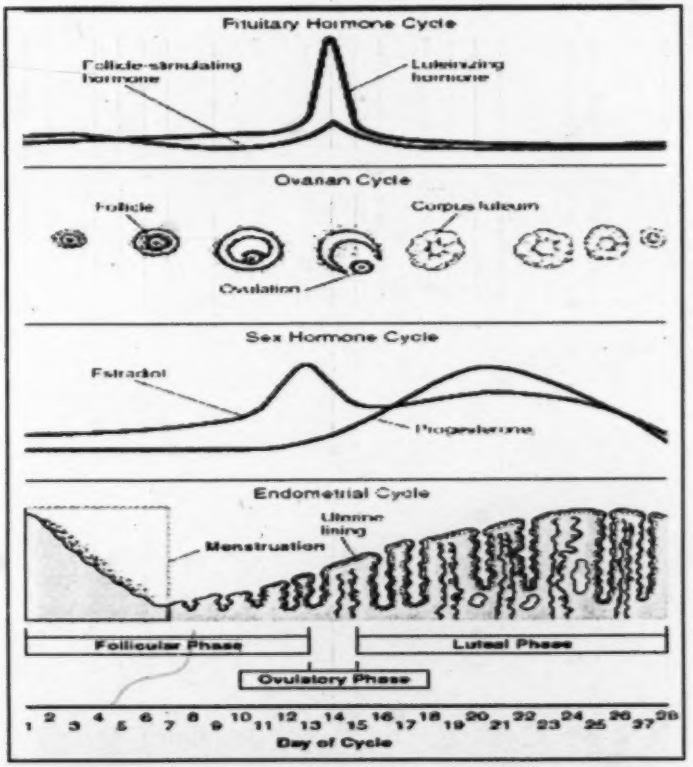

Figure 1.1 *The Human Menstrual Cycle* (From The Merck Manual of Medical Information -- Home Edition, Merck & Co., Inc., Whitehouse Station, NJ, USA, 1999; Website: http://www.merck.com/pubs/mmanual_home/ch232a.htm).

During menses, E_2 and progesterone levels are very low due to low steroid synthesis by immature follicles following regression of the corpus luteum (from the previous menstrual cycle). In response to the removal of negative feedback, plasma FSH levels are elevated while LH levels are low. FSH acts on a cohort of recruited follicles and plasma E_2 levels rise slightly between days 3 and 7, while the dominant follicle (to become a mature oocyte, the Graafian follicle) is selected between days 5 and 7. This follicle begins to grow and increases its steroidogenic activity. Between days 8 and 10, plasma E_2 levels rise sharply, reaching peak levels above 200 $pg \cdot ml^{-1}$ on the 12th day, the day before the LH surge (Rhoades & Tanner, 1995).

During the early follicular phase, LH pulsatility is of high frequency and low amplitude. As E_2 levels rise, LH pulse amplitude remains constant while the pulse frequency continues to increase. This results in a small, gradual rise in mean plasma LH level, which supports follicular steroidogenesis. At the mid- to late-follicular phase, E_2 suppresses FSH release at which time atresia of the non-dominant follicles occurs (Rhoades & Tanner, 1995).

The LH surge at mid-cycle lasts 24-36 hours and triggers the release of an ovum from the mature follicle. For this surge to occur, E_2 must be maintained at a critical concentration (approximately 200 $pg \cdot ml^{-1}$) for a sufficient duration of 36-48 hours. Prevention of the E_2 rise or a rise that is too small or too short eliminates the LH surge. Therefore, even though E_2 exerts a negative feedback on LH release most of the time, positive feedback by E_2 is required to generate the LH surge at mid-cycle. Furthermore, there is a small, but distinct rise in progesterone before the LH surge. Some believe that this rise is important for

augmenting the LH surge and, together with E_2, promoting a surge in FSH before ovulation (Rhoades & Tanner, 1995).

The remains of the follicle evolve into a corpus luteum. As the corpus luteum matures, progesterone and E_2 are secreted to prepare the endometrium for implantation of a fertilized egg. During the luteal phase, both progesterone and E_2 reach their highest concentrations on days 20-23, about 1 week after ovulation. Following the demise of the corpus luteum on days 24-26, E_2 and progesterone levels drop sharply. This results in withdrawal of support from the uterine endometrium and begins to shed, in which case menstruation begins within 2-3 days. This reduction in ovarian steroids acts centrally to remove feedback inhibition, whereby increasing the level of FSH and a new cycle is initiated (Rhoades & Tanner, 1995).

3. Menstrual Patterns

a) Eumenorrheic/Cyclic/Normal/Regular

Universally accepted definitions of various menstrual patterns are lacking and therefore discrepancies exist in what is considered a 'normal' length of a menstrual cycle. Loucks & Horvath (1985) prefer to use the terms *eumenorrheic*, *regular* and *cyclic* interchangeably, to refer to women whose menstrual cycles recur consistently at intervals from 25-38 days. Loucks & Horvath (1985) regard 'regularity' more important than interval length when classifying a woman's menstrual pattern. For example, they would classify a woman as 'eumenorrheic,' whose menstrual cycles were consistently 39 or 40

days in length. Nonetheless, 'normal' menstrual cycles have been classified as 23-35 days (Otis, 1992) and 21-36 days (Prior, 1990) in length. Whereas, Constantini & Warren (1994a) would consider a woman 'eumenorrheic' if her menstrual cycles were consistently between 21-36 days. For the purpose of this study, 'eumenorrheic' was defined as having a documented average menstrual cycle length of 24 to 37 days, for the previous 3 menstrual cycles .

b) Luteal Suppression (Shortened Luteal Phase & Insufficient Luteal Phase)

Luteal suppression refers to a reduction in the length of the luteal phase or a reduction in progesterone levels during the luteal phase of a menstrual cycle (Loucks, 1996). A *shortened luteal phase* is defined as a luteal phase < 9 days, whereas an *inadequate luteal phase* is defined as a luteal phase ≥ 9 days, with an insufficient amount of progesterone produced by the corpus luteum (Beitens et al., 1991). Women who suffer from luteal suppression, typically have no recognizable symptoms, as their menstrual cycles usually remain the same length (Otis, 1992). The only way to detect luteal suppression is by serial sampling of progesterone in blood, urine or saliva throughout a menstrual cycle (Loucks, 1996).

c) Anovulatory & Oligomenorrheic/Irregular

Anovulation is a menstrual irregularity in which the release of an ovum from the mature follicle is lacking. Anovulaton may occur: a) with an E_2 and an LH surge at mid-cycle without subsequent egg release; b) with an E_2 but no LH surge at mid-cycle; and c) with no mid-cycle increase in either E_2 or LH (Prior, 1990). Women with this dysfunction may have one of several different patterns of irregular bleeding, from

fewer than 21 days (Otis, 1992; Dueck et al., 1996; Shangold et al., 1990) to cycle lengths up to 35 to 150 days (Otis, 1992; Dueck et al., 1996). The latter pattern is referred to as *oligomenorrhea.*

Controversy exists in the classification of oligomenorrhea. Loucks & Horvath (1985) use the terms *oligomenorrheic* and *irregular* interchangeably, in reference to a woman whose menstrual cycles occur inconsistently at intervals from 39 to 90 days. Whereas, oligomenorrhea has been defined as menstrual cycles occurring at intervals greater than 36 days (Constantini & Warren, 1994a; Nattiv, 1994; Prior, 1990) and cycles that recur up to 4 months (Shangold et al. 1990).

d) Amenorrheic/Acyclic

The terms *amenorrheic* and *acyclic* are used interchangeably and refers to the cessation of menstrual function (Loucks & Horvath, 1985). There are two types of AMEN in which there is complete absence of follicular development, ovulation, and luteal function (Loucks, 1996). *Primary AMEN* indicates that menarche (first menstrual period) has been delayed beyond the age of 16 (Loucks & Horvath, 1985; Constantini & Warren, 1994a). *Secondary AMEN* signifies that menses has ceased sometime after menarche (Loucks, 1996). Definitions used for secondary AMEN vary from the absence of menstruation for at least 90 days (Loucks & Horvath, 1985), 3 months (Nattiv et al., 1994), 6 months (Harber et al., 1998; Loucks et al., 1992; Myerson et al., 1991), or for up to 12 months (Harber et al., 1991), or less than 2 menses (Baer, 1993; Friday et al., 1993) or 3 menses (Wilmore et al. 1992) per year.

4. Energy Availability

Energy availability is defined as the amount of energy consumed minus the amount of energy expended during exercise (ie. physical activity) (Loucks & Callister, 1993). The energy availability hypothesis is based on the premise that when energetically challenged, priorities are established such that functions that are essential for individual survival (eg. thermoregulation, locomotion, cellular maintenance) are maintained, at the expense of other activities that can be delayed (eg. reproduction, growth, adipose tissue) (Wade & Schneider, 1992).

There is an evolutionary and physiological rationale for the energy availability hypothesis to explain reproductive disorders in physically active women. Is it believed that the avoidance of follicular development, endometrial proliferation, hormone synthesis, increased luteal phase thermogenesis, and other anabolic and catabolic processes of the menstrual cycle will contribute to a reduction in energy utilization when energy availability is low, which will therefore spare the body's protein content and promote individual survival (Loucks & Callister, 1993).

5. Fat Mass, Fat Free Mass and Lean Body Mass

a) Fat Mass (FM)

Fat mass is defined as all extractable lipids from adipose tissue and other tissues in the body (Heyward & Stolarczyk, 1996).

b) Fat Free Mass (FFM)

Fat free mass is the weight of all tissues in the body minus the extractable fat. It is chemically composed of water, protein and bone mineral. The '2-component body composition model' assumes the body is composed of FM and FFM, and can be determined through hydrodensitometry (Heyward & Stolarczyk, 1996).

c) Lean Body Mass (LBM)

In contrast to FFM (which contains no lipids) *lean body mass* includes a small amount of essential lipids (2-3 % in males and 5-8% in females) and therefore these two terms should not be used interchangeably (Lohman, 1992). Note, in all of the studies of Loucks and her coworkers (which have examined the effects of energy availability on thyroid metabolism and LH pulsatility), energy availability was based on kcal·kg LBM^{-1}·day^{-1}. However, Loucks and her coworkers determined the subjects' body composition using hydrostatic weighing or skinfolds (ie. 2-component body composition model) where energy availability should be expressed as kcal·kg FFM^{-1}·day^{-1}.

6. Hypothalamic-Pituitary-Thyroid Axis

Thyroid hormone secretion is regulated by a feedback system between hypothalamic thyrotropin-releasing hormone (TRH), anterior pituitary thyroid-stimulating hormone (TSH), and the thyroid gland hormones: 3,5,3',5'-tetraiodothyronine or thyroxine (T_4) and T_3. T_4 is produced by the thyroid gland,

whereas T_3 is predominantly produced by the conversion of T_4 at extra-thyroidal sites (eg. liver, kidney) and to a lesser extent produced by the thyroid gland (Felig et al., 1995).

Most circulating thyroid hormones are bound to the plasma proteins: thyroxine-binding globulin (TBG), albumin and transthyretin (TTR) (formerly called thyroxine-binding prealbumin; TBPA). Less than 1 % of T_4 and T_3 in blood, is in the free form (ie. fT_4 and fT_3), which is in equilibrium with the large protein-bound fraction. It is this small amount of free thyroid hormone that interacts with target cells and determines the hormones' biological activity (Rhodes & Tanner, 1995). Furthermore, T_3 is considered the major physiologically active form of the thyroid hormones, due to the fact that 70 to 90 % of T_3 is derived from T_4 (Leonard & Koehrle, 1996) and T_3 is approximately 3 to 4 times more potent than T_4 in eliciting metabolic responses (Lerman, 1953).

There are 2 general types of deiodination reactions (Figure 1.2). The removal of the 5'- (or 3'-) iodine atom from the phenolic ring (ie. the outer ring) of iodothyronines, is designated as *5'-deiodination*. Whereas, the removal of the 5- (or 3-) iodine atom from the tyrosyl ring (ie. the inner ring) of iodothyronines, is designated as *5-deiodination*.

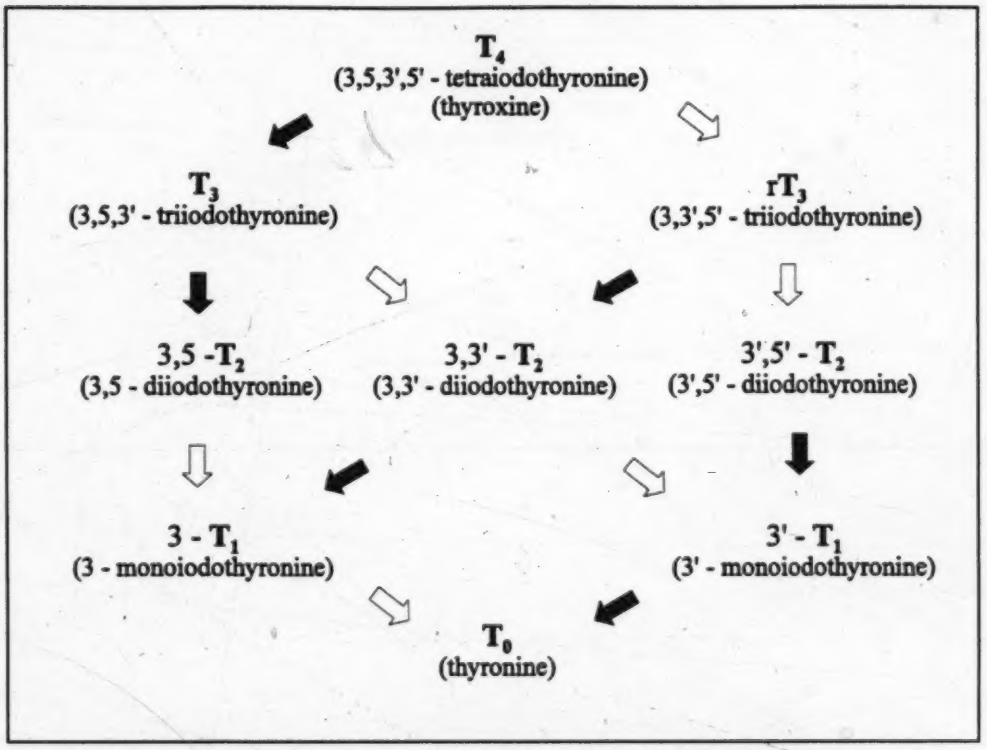

Figure I.2 Pathways of sequential monodeiodination cascade of thyroxine and other iodothyronines. Black arrows illustrate 5'-(or 3'-) deiodination routes and white arrows illustrate 5-(or 3-) deiodination routes. (Köhre J, M Auf'mkolk, M Spanka, K Irmscher, V Cody, RD Hesch: *Iodothyronine deiodinase is inhibited by plant flavonoids.* IN: Cody V, E Middleton Jr., JB Harborne, eds. *Progress in clinical and biological research.* 1986; 213:359).

These pathways generate all of the iodothyronines shown in the deiodination cascade, from the fully iodinated form (T_4) to the iodine-free thyronine molecule (T_0). The deiodination of T_4 occurs by stepwise, sequential removal of iodine atoms from either the phenolic ring or tyrosyl ring. About 70 % of the T_4 secreted daily is deiodinated to yield equal amounts of T_3 and rT_3. As noted earlier, 70 to 90 % of the daily production of T_3 originates from extra-thyroidal deiodination from T_4, with the rest derived from

the thyroid. In contrast, 95 to 98 % of the rT_3 produced daily is generated by deiodination, and only a minute amount of rT_3 is derived from the thyroid. Subsequently, deiodination of T_3 and rT_3 yields the 3-diiodothyronines (T_2), which are: 3,5-T_2; 3,3'-T_2; and 3',5'-T_2. The three T_2s combined daily production equates to approximately 67 % of the total production of T_3 and rT_3, and therefore indicates that nondeiodinative pathways account for about 33 % of the metabolism of iodothyronines (Leonard & Koehrle, 1996).

There appears to be at least 3-enzymatic iodothyronine-deiodinating pathways. These pathways are:

a) Type I 5'- (or 3'-) Deiodinase

Type I 5'-deiodinase catalyses deiodination of T_4 to T_3, and rT_3 to 3,3'-T_2 and catalyses diiodothyronines and monoiodothyronines which have phenolic ring iodines (Leonard & Koehrle, 1996). Type I 5'-deiodinase has its highest levels of activity in the thyroid, kidney and liver (Leonard & Koehrle, 1996), and it appears that most of the circulating T_3 is derived via hepatic deiodination of T_4 by this pathway (Kaplan, 1984).

b) Type II 5'- (or 3'-) Deiodinase

The second *5'-deiodinase* pathway is known as *Type II 5'-deiodinase* and it also catalyses deiodination of T_4 to T_3, and rT_3 to 3,3'-T_2 (Leonard & Koehrle, 1996). It appears that Type II 5'-deiodinase generates T_3 for local use in organs such as the brain, pituitary and brown adipose tissue (Kaplan, 1984; Leonard & Koehrle, 1996).

c) Type III 5- (or 3-) Deiodinase

The 3rd pathway in which deiodination of iodothyronines occurs, is at the 5- or 3- position (on the tyrosyl ring). This pathway is one of the major routes for T_3 degradation (to the inactive $3,3'\text{-}T_2$) and inactivation of T_4 (to rT_3) (Kaplan, 1984; Leonard & Koehrle, 1996). In addition, this pathway appears in almost every tissue except the anterior pituitary, and it has high levels of activity present in the liver, brain and placenta (Leonard & Koehrle, 1996).

The thyroid hormones have many physiological actions within the body. These roles include: 1) the development of the central nervous system; 2) normal body growth (eg. stimulate the expression of the gene for GH and promote calcification of growth plates of bones); 3) regulation of the basal energy economy of the body; 4) stimulation of intermediary metabolism; and 5) regulation of their own secretion (Rhodes & Tanner, 1995).

7. 'Low-T_3 Syndrome' vs 'Lowered T_3 State'

The term *Low-T_3 syndrome* has been used by Loucks & Callister (1993) and Loucks & Heath (1994a) indicating that reductions in T_3 and fT_3 levels with elevations in rT_3 levels have occurred. However, these changes in circulating thyroids, as a result of 4-days of reduced energy availability, remained within a normal range. Since these are not suggestive of a pathological state, using the term 'syndrome' may not be appropriate in defining this occurrence. A more appropriate term may be 'lowered T_3 state' indicating that changes have occurred in these metabolic markers, without inferring pathology.

Reduced activity of deiodinase at extra-thyroidal sites accounts for decreased conversion of T_4 to T_3 (Vagenakis et al., 1977; LoPresti et al., 1991). The rise of serum rT_3 values in 'lowered T_3 state' appears to be a consequence of a decreased rT_3 clearance, as peripheral conversion of T_4 to rT_3 remains unchanged and serum T_4 levels are usually maintained in the normal range (LoPresti et al., 1991).

8. Thyroid Threshold

When referring to the thyroid data, the term *threshold* refers to a statistically significant difference between treatment groups using the LSD post-hoc test when appropriate.

9. Ketones

At times when the body's CHO content is extremely low (eg. during starvation, extremely low CHO diets, during prolonged exercise without sufficient CHO ingestion), lipolysis (the breakdown of stored fat into glycerol and fatty acids) is accelerated as the body attempts to meet its energy needs (Houston, 1995). During these times of CHO deficiency, fat oxidation is incomplete, acetyl-CoA accumulates, and the liver converts acetyl-CoA molecules to *ketones* (or *ketone bodies*), which are released into the blood. This conversion process is called *ketogenesis*. There are 3 different ketone bodies which are all formed from acetic acid, these include: acetoacetic acid, β-hydroxybutyric acid and acetone (Marieb, 1998). These ketone bodies are released from the liver and are used by tissues as a form of fuel. For example, the main ketone body from the liver, β-hydroxybutyrate is released during exercise or starvation, and circulated to

the active skeletal muscle where it is taken up, converted to 2 acetyl-CoA molecules, and used in mitochondrial respiration (Robergs & Roberts, 1997). Furthermore, glucose is normally the major source of energy in the brain. However, during times of CHO deficiency, ketone bodies become an important source of energy for the brain (Zubay, 1995).

When ketones accumulate in the blood faster than they can be used by tissue cells for fuel, *ketosis* results and large amounts of ketones are excreted from the body in the urine (ie. *ketonuria*). An outcome of ketosis is *metabolic acidosis* (or *ketoacidosis*). This is the result of the body's buffering system unable to keep up with the formation of hydrogen ions (H^+) when acetoacetate is formed, and the blood pH can drop to dangerously low levels. In addition, an individual's breath may smell 'fruity' as acetone diffuses out of the pulmonary capillaries and into the alveoli of the lungs, and breathing may become rapid as the respiratory system tries to blow off blood carbonic acid as CO_2, in an effort to force the blood pH upwards (Marieb, 1998).

10. Respiratory Quotient, Respiratory Exchange Ratio and Nonprotein RQ/RER (Table I.2)

The ratio between the number of moles of O_2 used to metabolize carbohydrate and fat and the number of moles of CO_2 produced, is traditionally called the *respiratory quotient (RQ)*. The *respiratory exchange ratio (RER)* is calculated the same way, but the conditions in which CO_2 is produced differs. The RQ is used to indicate cellular respiration and therefore the O_2 and CO_2 results from the catabolism of food.

Conversely, RER is used when $\dot{V}O_2$ and $\dot{V}CO_2$ are measured from ventilated air resulting from external respiration at the lungs (Robergs & Roberts, 1997).

If the body metabolized only CHOs and fats (and no protein), then the RQ would represent the proportional use of these 2 energy nutrients, as there is only a negligible loss of energy from the digestion of CHOs and fats. For example, an RQ of 1.00 indicates that only CHOs are being metabolized, and an RQ of 0.70 indicates that only fat is being metabolized in the cell. Any ratio between these 2 values, indicates the relative combinations of these 2 foods. However, due to protein being a part of the diet, it too is metabolized in the body and must be taken into consideration. Unlike CHO and fat, when protein is metabolized, some energy is lost as nitrogen and a small amount of sulphur residue are excreted in the urine and feces. Due to this loss of energy, special consideration must be taken when using RQ to determine the type of energy nutrient being metabolized. This is of added significance when using RQ to assign the caloric value to each litre of oxygen that the body is using. For this reason, *nonprotein RQ* (or *nonprotein RER*) values have been determined by subtracting the O_2 required and the CO_2 produced when protein is oxidized from the total $\dot{V}O_2$ (Foss & Keteyian, 1998). Note, in most cases the gross metabolic RQ calculated without the additional measures to determine protein utilization, introduces only minimal error, due to the usual small contribution of protein for energy metabolism (McArdle, Katch & Katch, 1991).

When sampling air from the lungs (ie. using indirect calorimetry), the ratio of $\dot{V}CO_2 \cdot \dot{V}O_2^{-1}$ can be modified, which is unrelated to the cellular production of CO_2 from the catabolic pathways of CHO, fat or

protein. Therefore, the assumption of equality between RQ and RER cannot be made under certain conditions (Foss & Keteyian, 1998; Robergs & Roberts, 1997). These circumstances are as follows:

a) Metabolic Acidosis

The production of CO_2 can not exceed the consumption of O_2 within the cell, and therefore the maximal RQ value that can occur is 1.0 (when only CHOs are metabolized). However, during metabolic conditions that increase acid production (eg. ketosis and during intense exercise when lactic acid is produced), the added CO_2 produced from the buffering of acid in the body increases $\dot{V}CO_2$, independent of $\dot{V}O_2$, and therefore RER values can exceed 1.0.

b) Hyperventilation

This phenomenon results in higher-than-normal quantities of CO_2 being eliminated through the lungs, without similar increases in $\dot{V}O_2$. This effect therefore produces an inflated RER value.

c) Excess Post-Exercise $\dot{V}O_2$

During the recovery period from exercise, $\dot{V}CO_2$ decreases rapidly. Conversely, $\dot{V}O_2$ declines, but remains elevated above pre-exercise values for several minutes. Consequently, RER values may decrease to levels below resting values.

d) Non-Steady State Conditions

During increasing intensity of exercise, it takes time for the $\dot{V}O_2$ to increase to a level that accounts for the ATP produced by aerobic metabolism. During the time that ATP is produced from alternative sources (ie. creatine phosphate hydrolysis and glycolysis), RQ values will be falsely elevated. Therefore,

steady-state conditions (which should be reached in approximately 3 minutes if the exercise intensity is not too high) should be achieved prior to determining RER.

Table I.2

Nonprotein RER-Caloric equivalents and % calories provided by glucose and fatty acids

Nonprotein respiratory exchange ratio (RER)	kcal·L^{-1} of O_2 consumed	Percentage of calories derived from:	
		Glucose	Fatty acids
0.70	4.851	0.0	100.0
0.75	4.904	16.8	83.2
0.80	4.962	34.5	65.5
0.81	4.974	38.0	62.0
0.82	4.985	41.4	58.6
0.83	4.997	44.9	55.1
0.84	5.008	48.3	51.7
0.85	5.020	51.7	48.3
0.86	5.032	55.1	44.9
0.87	5.043	58.5	41.5
0.88	5.055	61.9	38.1
0.89	5.066	65.3	34.7
0.90	5.078	68.6	31.4
0.91	5.089	71.9	28.1
0.92	5.101	75.3	24.7
0.93	5.112	78.6	21.4
0.94	5.124	81.8	18.2
0.95	5.136	85.1	14.9
0.96	5.147	88.4	11.6
0.97	5.159	91.6	8.4
0.98	5.170	94.8	5.2
0.99	5.182	98.0	2.0
1.00	5.189	100.0	0.0

From Péronnet, F. & D. Massicotte (1991).

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CHAPTER II

LITERATURE REVIEW

INTRODUCTION

Athletic women participating in sports which emphasize a lean physique or a low body weight (eg. distance running, gymnastics, figure skating, ballet dancing) are more likely to adopt weight loss strategies such as fewer dietary calories or an increase in physical activity or the combination of both. As a result, they may put themselves at risk for nutritional deficiencies (Deuster et al., 1986), menstrual irregularities (De Souza et al., 1998; Loucks et al., 1994b; Loucks et al., 1994c; Loucks et al., 1995; Williams et al., 1995; Loucks & Heath, 1998a; Loucks et al., 1998b), cardiovascular disease (Friday et al., 1993), stress fractures, premature osteoporosis (Drinkwater, 1989; Nelson et al., 1986), the development of eating disorders (Sundgot-Borgen, 1993; Sundgot-Borgen, 1994) and slower recovery times following intense exercise (Harber et al., 1998). Furthermore, exercising women who experience reductions in metabolic rate (Myerson et al., 1991), may also increase their susceptibility for increased fat mass once training is reduced or terminated (Baer, 1993). Although exercise is performed on a regular basis and often for long durations, additional energy expenditures are often not sufficient to maintain "ideal" or "optimal" weight; thus, energy intakes are often sacrificed. Numerous cross-sectional studies have found that weight stable athletic women report energy intakes similar to those of sedentary EUM women (Beidleman et al., 1995; Broocks et

al., 1990; Dahlstrom et al., 1995; Deuster et al., 1986; Drinkwater et al., 1984; Edwards et al., 1993; Harber et al., 1998; Loucks et al., 1992; Mulligan & Butterfield, 1990; Myerson et al., 1991; Perry et al., 1996; Schwartz et al., 1981; Schweiger et al., 1988; Snead et al., 1992; Watkin et al., 1991; Wilmore et al., 1992). In addition, some researchers believe that inadequate nutrition plays a role in reproductive dysfunction, as physically trained AMEN women have reported consuming significantly fewer calories then their EUM counterparts (Baer, 1993; Kaiserauer et al., 1989; Marcus et al., 1985; Nelson et al., 1986).

The suspicion that nutrition plays a role in reproductive function (De Souza et al., 1998; Loucks, 1996) is strengthened by repeated discoveries of low T_3 levels (a sign of energy deficiency), in physically trained AMEN but not in physically trained EUM women (Baer, 1993; Harber et al., 1998; Loucks et al., 1992; Marcus et al., 1985; Myerson et al., 1991). It has been proposed that the decline in this metabolically active thyroid hormone may be part of an adaptive syndrome in response to an energy deficient state, to conserve energy and maintain stable weight (Baer, 1993; Loucks et al., 1992; Marcus et al., 1985; Myerson et al., 1991). Furthermore, due to T_3 being the major metabolically active hormone, it has been suggested that resting metabolic rate (RMR) may also be reduced. This relationship has been documented in physically trained AMEN women, who have significantly lower T_3 levels and RMRs compared to physically trained EUM and EUM sedentary women (Myerson et al., 1991). However, the only other study to determine both T_3 levels and RMRs in AMEN and EUM athletes and sedentary EUM women, did not find this relationship of lower T_3 levels and RMRs in AMEN athletes (Wilmore et al., 1992).

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discussion to appraise the literature in hope of defining the functional relationship between energy availability and thyroid metabolism. If experimental results indicate that the relationship between energy availability and thyroid hormones is proportional, then dietary compensation for exercise may be a possible strategy to prevent detrimental changes in thyroid hormones. Identification of the level of energy availability at which changes occur in thyroid hormones, may assist in determining the requisite amount of energy necessary to maintain thyroid hormone levels, and perhaps reproductive function as well. Moreover, if circulating thyroid hormones are an indicator of energy availability, then clarification of this relationship is essential for the development of weight control programs for women, whereby reproductive function will not be altered or sacrificed.

THYROID HORMONES AND DIET

Numerous studies have been performed to determine the effects of caloric restriction, fasting and the macronutrient (ie. protein, CHO, fat) content of one's diet on thyroid metabolism. Most studies have been conducted on sedentary, obese males (Azizi, 1978; Carlson et al., 1977; Vagenakis et al., 1975; Vagenakis et al., 1977), sedentary obese women (LoPresti et al., 1991; Vagenakis et al., 1975; Vagenakis et al., 1977), with only a few studies examining the effects of diet on thyroid metabolism in sedentary normal weight males (Palmblad et al., 1997) and sedentary normal weight women (Loucks & Heath, 1994c). Furthermore, additional studies have not indicated the sex of their sedentary, obese subjects (O'Brian et al., 1980; Spaulding et al., 1976) or sedentary, normal weight subjects (Serog et al., 1982).

I. Gender and Thyroid Metabolism

Most research has found that there are no differences in pituitary-thyroid function in women and men in regard to thyroid secretion, extra-thyroidal thyroid hormone metabolism, or serum thyroid hormone-binding proteins, and therefore serum T_4, T_3, and TSH concentrations appear to be similar between the sexes. While serum thyroxine-binding globulin (TBG) concentrations are increased by excess estrogen and decreased by excess androgen, serum TBG concentrations are similar in men, premenopausal and postmenopausal women, despite the differences in gonadal steroid hormone production among these groups (Felig et al., 1995). In contrast to these findings, Azizi (1978) reported greater decreases in T_3 and fT_3 and greater increases in T_4 and fT_4 in men versus women following a 4-day fast. Conversely, significant increases in the metabolically inactive hormone - rT_3, were observed in both the men and women of the same magnitude. The author noted that the higher level of estrogen in obese women may be responsible for the difference between men and women, since E_2 increases the peripheral conversion of T_4 to T_3.

2. Calories vs Macronutrients

Research indicates that the amount of CHO in one's diet may alter the peripheral conversion of T_4 to either T_3 or rT_3, while the protein and fat content of one's dietary intake is less involved in regulating the level of thyroid hormones (Azizi, 1978; Serog et al., 1982; Spaulding et al.,1976). For example, after a 4-day fast, subjects were refed for 4 days with a 800 kcal·day^{-1} mixed or exclusively CHO diet, in which T_3

levels returned to baseline, while T_3 levels continued to decrease when the diet contained only protein and fat (Azizi, 1978). Furthermore, Serog et al. (1982) had subjects consume a low (10%), moderate (55%) and high (75%) CHO isocaloric (2,800 kcal·day^{-1}) diet for one week and noted that the low CHO diet decreased T_3 and increased rT_3. The investigators hypothesized that although CHO was not a unique trigger in the process, perhaps CHO acts only below a threshold or may be in relationship with the energy intake level (Serog et al., 1982).

With this threshold concept in mind, the effects of caloric and CHO restriction on thyroid metabolism in sedentary obese patients has been conducted. Studies have shown that decreases in T_3 levels occur only when dietary energy intake falls below a particular amount, with the CHO content of the diet influencing this value (O'Brian et al., 1980; Spaulding et al., 1976). Spaulding et al. (1976) found that T_3 levels fell when energy intake was reduced to 800 kcal·day^{-1} if CHO content was $<$200 kcal·day^{-1}; however, when energy intake was reduced to 600 kcal·day^{-1} or less, T_3 levels fell regardless of the CHO content (O'Brian et al., 1980). The levels of rT_3 have been found to rise with low energy intakes, independent of dietary CHO content. Elevations of rT_3 occurred at reduced energy intakes of 600 kcal·day^{-1} or less (O'Brian et al., 1980) and were eliminated by energy intakes of 800 kcal·day^{-1} in diets containing no CHO, only CHO and an isocaloric mixed diet (Spaulding et al., 1976).

Recently, the concept of energy availability has been examined in sedentary normal-weight menstruating women in a series of 4-day studies, referred to the EXCALIBUR experiments (Loucks & Callister, 1993; Loucks & Heath, 1994a; Loucks & Heath, 1994b; Loucks et al., 1994c; Loucks et al., 1995).

All of these experiments, except for Loucks and Heath (1994c), have established specific energy availability levels through the implementation of varying degrees of caloric intake and energy expenditure using aerobic exercise. Conversely, Loucks and Heath (1994c) manipulated energy availability through specific caloric intakes and did not implement an exercising component. Dietary energy intakes (and thus energy availabilities) were set for one group of women who were instructed to continue their habitual caloric intakes of 1940 kcal·day^{-1} (45 kcal·kg LBM^{-1}·day^{-1}) while another group were restricted to only 440 kcal·day^{-1} (10 kcal·kg LBM^{-1}·day^{-1}). Caloric intakes from the restricted group were similar to those implemented by O'Brian et al. (1980) with results (ie. a decrease in T_3 levels) leading some to believe at that time, that there was a threshold relationship between caloric intake and thyroid hormones. Although the main purpose of Loucks & Heath's (1994c) experiment was to determine if different energy availability levels would effect LH pulsatility, T_3 levels were also assessed. Significant decreases in T_3 levels (20%), decreases in LH pulse frequency (23%) and increases in LH pulse amplitude (40%) were observed in the dietary energy restricted group. These pulse characteristics were similar to those noted in competitive triathletes and runners, whose menstrual cycles were symptomatically indistinguishable from those of sedentary women (ie. occurring at regular intervals of 26 to 32 days). However, subclinical menstrual changes were found in these athletes who displayed luteal phases that were 2 days shorter and progesterone levels that were reduced almost 50% (Loucks et al., 1989). Thus, Loucks & Heath's (1994c) 4-day experiment produced results similar to those seen in physically trained women experiencing luteal

suppression. Therefore, low energy availability was associated with both LH pulsatility and thyroid hormone levels.

3. Acute vs Chronic Dietary Manipulation

Acute studies that have manipulated diet over a span of four to 14 days have provided somewhat consistent results with no change in T_4, a decrease in T_3, and an increase in rT_3 (O'Brian et al., 1980; Serog et al., 1982; Vagenakis et al., 1975). However, Azizi's (1978) 4-day fast found T_4 increased and Palmblad et al. (1977) 11-day fast found that T_4 had decreased. Moreover, the majority of studies have found that T_3 decreases during very low caloric intakes or fasting and that an increase in rT_3 is produced (Azizi, 1978; Carlson et al., 1977; LoPresti et al., 1991; O'Brian et al., 1980; Palmblad et al., 1977; Serog et al., 1982; Vagenakis et al., 1975). Although, a 2-week 800 kcal·day^{-1} diet implemented by Spaulding et al. (1976) found no changes in rT_3, even with varying amounts of macronutrients in the diets consumed.

These exceptions may be explained by the differences in the length and severity of dietary manipulation and the frequency of blood sampling for thyroid hormone assessment. For example, restrictive diets lead to significant decreases in serum T_4, fT_3 (Carlson et al., 1977) and T_3 (Carlson et al., 1977; O'Brian et al., 1980) and an increase in serum rT_3 (Carlson et al., 1977; O'Brien et al., 1980); however, these values tended to return toward pre-diet levels as the experiment continued beyond 2 weeks of fasting (O'Brian et al., 1980) and 3 weeks of dietary restriction (Carlson et al., 1977). These results indicate the importance of assessing blood at regular intervals throughout a study, as there does not appear

to be consistent changes in increasing or decreasing amounts of thyroid hormone concentrations over a long period of time. The effects of dietary manipulation on thyroid hormones requires further clarification with standardized length and degree of restriction in the diet.

THYROID HORMONES AND EXERCISE

Studies examining the relationship between thyroid hormones and exercise in females have been conducted on trained normal weight women (Boyden et al., 1982; Boyden et al., 1984; Hohtari et al., 1987) and untrained obese (Caron et al., 1986; Krotkiewski et al., 1984) women. Research indicates that alterations in thyroid function may occur with repeated exercise. Initial findings by Boyden et al. (1982) suggest that moderately trained EUM women joggers who increased their training to 48 $km \cdot wk^{-1}$, may develop mild thyroidal impairment as decreases in both T_3 and rT_3 were noted, with no change in T_4. The authors suggested that the mechanism of thyroid adaptations may be different in women runners than during caloric deprivation, as an increase in rT_3 did not occur in the runners. Since both T_3 and rT_3 levels decreased in the subjects, while T_4 levels were unchanged, the authors noted that it is tempting to speculate that endurance training induces decreased 5'- and 5-deiodinase activity and/or increased metabolic clearance of T_3 and rT_3. However, when the subjects increased their mileage to 80 $km \cdot wk^{-1}$, T_4 and rT_3 increased above pre-exercise levels, and T_3 levels increased to pre-exercise levels (Boyden et al., 1984). These results therefore make it difficult to speculate the effects that endurance training has on thyroid metabolism. One should note however, that for both of Boyden et al.'s studies (1982; 1984), there were no

changes in body weight of the subjects. However, the subjects lean body weight increased progressively (ie. from baseline to 48 km·wk^{-1} to 80 km·wk^{-1}) and their fat weight and relative % body fat decreased progressively, suggesting that the subjects were in an energy deficient state during both volumes of training and to an even greater degree when running 80 km·wk^{-1}, which may be a cause for contradictory results between the two studies.

Another study examining the effects of different training intensities and seasons on EUM competitive runners (ie. autumn was their 'light' intensity season versus spring being their 'hard' intensity season) with an average running mileage of 42 km·wk^{-1}, and EUM recreational joggers running 15-60 km·wk^{-1}, were compared to EUM sedentary non-obese women (Hohtari et al., 1987). The runners displayed significantly lower E_2 levels compared to their sedentary counterparts during both training seasons, whereas the joggers E_2 levels were no different to their sedentary controls. Lower T_4 and fT_4 levels were observed in the competitive runners in both seasons, as well as reductions in T_3 during the light season, compared to their controls. It should be noted that the runners % body fat decreased from the light to hard training season (ie. autumn to spring), whereas there were no significant % body fat changes found in the other groups of women. This therefore makes it difficult to explain the reduction in T_3 during the light season, as one would expect a decrease during the hard season when the runners % body fat decreased. In addition, TBG levels were also reduced in the runners in the luteal phase of their menstrual cycle in both seasons. Similar results were seen in the joggers, however thyroid hormone changes were less pronounced. Furthermore, independent of training, TSH, T_4, fT_4, T_3 and TBG levels were slightly higher in the spring

compared to the autumn. The authors noted that the reduced T_4 levels in the runners may have been due to lower TBG levels, rather than due to the direct effect of training. Moreover, Hohtari et al. (1987) hypothesized that due to the runners displaying lower E_2 levels compared to their sedentary controls, this could possibly explain the reduced TBG levels.

Inconsistent results have been reported in obese, sedentary women beginning an aerobic exercise training program. No changes in T_3, rT_3 (Caron et al., 1986), T_4 (Caron et al., 1986; Krotkiewski et al., 1984) and TSH (Caron et al., 1986) have occurred whereas reductions in T_3 and increases in rT_3 have been reported (Krotkiewski et al., 1984). It should be noted however, that body weight decreased (Caron et al., 1986) and increased (Krotkiewski et al., 1984), suggesting that these women were at different energy status'. Therefore, when examining results from studies which have looked at the effects of exercise on thyroid metabolism, these results may have been confounded by the independent variable of caloric intake, and subsequent comparisons between studies may be very difficult.

THYROID HORMONES, DIET AND EXERCISE

Few studies have examined the relationships between thyroid hormones, diet and exercise in women. If in fact the level of energy availability leads to changes in thyroid hormones, studies risk confounding their results by not quantifying both caloric intake and energy expenditure to determine the energy status of their subjects. One of the first studies that attempted to control for these variables was completed by Mathieson et al. (1986). This study examined the effects of a very low caloric diet (530

kcal·day^{-1}) with a low and high CHO content (33% and 71%, respectively), in combination with continuous aerobic exercise for 28 days in obese untrained women. No change in T_4 levels were observed but an increase in rT_3 levels occurred within the first week, along with a decrease in T_3. Furthermore, the magnitude of T_3 response was significantly different between the two CHO diets. The low and high CHO diets caused T_3 levels to decrease by 34.6% and 17.9%, respectively. The authors noted that although the CHO content in the diet influenced the magnitude of fall in T_3 levels, RMR declined similarly for both dietary treatment groups. Moreover, Mathieson et al. (1986) suggested that the exercise may have counter-balanced the effects of the very low caloric diet on RMR, by increasing sympathetic activity.

Few studies have controlled caloric intake and exercise energy expenditure to precisely determine energy availabilities. Some have examined thyroid hormones (Loucks & Callister, 1993; Loucks & Heath, 1994a) while others have examined LH pulsatility (Loucks et al., 1994b; Loucks et al., 1995; Williams et al., 1995; Loucks & Heath, 1998a; Loucks et al., 1998b) to fixed energy availabilities. These studies examining thyroid hormones and LH have been performed to determine if there are similar energy availability thresholds, suggesting that the thyroid and reproductive axes may share a threshold dependence on energy availability (Loucks & Heath, 1994a).

In EXCALIBUR I, Loucks & Callister (1993) manipulated energy availability (ie. 8 vs 30 kcal·kg body wt^{-1}·day^{-1}) by means of altering dietary intake and exercise energy expenditure (with variations in: 1. volume - no exercise vs 1,300 kcal·day^{-1}; and 2. intensity - 40% vs 70% $\dot{V}O_{2max}$) in non-athletic women. Findings indicated that the energy cost of aerobic exercise induced 'lowered T_3 state'

within four days, and that this effect was prevented by increasing dietary energy intake to equal that of the energy cost of exercise. Beyond its impact on energy availability, exercise volume and intensity had no influence on thyroid metabolism. With these findings, Loucks & Heath (1994a) manipulated energy availability in EXCALIBUR II by having non-athletic women exercise at 70% $\dot{V}O_{2max}$ to expend 30 kcal·kg LBM^{-1}·day^{-1}. Depending on the assigned energy availability group (11.0, 19.0, 25.0 and 40.0 kcal·kg LBM^{-1}·day^{-1}), individual caloric intakes were allotted. EXCALIBUR II results indicated that decreases in T_3 and fT_3 occurred at a threshold of energy availability between 19.0 and 25.0 kcal·kg LBM^{-1}·day^{-1} and increases in fT_4 and rT_3 at a threshold of 11.0 and 19.0 kcal·kg LBM^{-1}·day^{-1}. These results indicate that it is not the energy intake or stress of exercise *per se*, but rather the absolute amount of energy available to the body that is the major determinant in metabolic responses. These results suggest that a partial dietary compensation for exercise energy expenditure might be sufficient to avoid reductions in T_3 levels and rising rT_3 levels . Therefore, if reproductive disorders in exercising women are eventually shown to be caused by a low energy availability, then these disorders might be prevented or reversed by dietary changes without reducing either the volume or the intensity of an exercise regime.

In addition to these findings, Williams et al. (1995) have also manipulated caloric intake and energy expenditure in trained, normal weight, menstruating women for 3 days during each of 3 consecutive months. The main focus of their experiment was to determine whether strenuous exercise with and without caloric restriction alters LH secretion, however, thyroid levels were also determined. Results indicated that women who consumed only 60% of the necessary calories to maintain weight, experienced significant

decreases in LH pulse frequency, in absence of an effect on LH peak amplitude. Despite decreased T_3 levels observed in each subject, these were not statistically significant. However, the subject who experienced the largest decrease in resting T_3 levels exhibited the largest decline in overall LH pulse frequency, and the subject that experienced the smallest decline in plasma T_3 experienced the smallest decrease in LH pulse frequency. These results suggest that LH pulses are dependent on energy status, and that the body responds to caloric restriction on an individual basis. Furthermore, it appears that LH frequency may have a greater sensitivity and be a better indicator of low energy availability compared to observing changes in T_3.

THYROID HORMONES AND ATHLETIC AMENORRHEA

Numerous studies have found significantly lower T_3 and fT_3 levels in physically trained AMEN women compared to either physically trained EUM women or EUM sedentary women (Baer, 1993; Harber et al., 1998; Loucks et al., 1992; Marcus et al., 1985; Myerson et al., 1991). These results suggest that AMEN athletes may have greater energy deficits than EUM athletes, and is expressed by menstrual dysfunction. Moreover, one would expect increased levels of rT_3 in AMEN athletes, as an increase in rT_3 is also a sign of energy deficiency, in the acute stages of low caloric or fasting studies (Azizi, 1978; LoPresti et al., 1991; Palmblad et al., 1977; Serog et al., 1982; Vagenakis et al., 1975). However, Loucks et al. (1992) found rT_3 levels lower compared to EUM athletes, whereas no differences were found by Myerson et al. (1991) and Wilmore et al. (1992). However, Myerson al et. (1991) indicated that there was a trend of lower rT_3 levels

in both AMEN and EUM runners. The authors suggested that both athletic groups may be voluntarily restraining their eating to remain thin.

Some physically active AMEN women consume fewer calories (Baer, 1993; Kaiserauer et al., 1989; Marcus et al., 1985; Nelson et al., 1986) and have higher exercise energy expenditures (Baer, 1993; Drinkwater et al., 1984) compared to their EUM peers. These findings suggest that AMEN athletes may be just below and EUM athletes just above the energy availability threshold, such that the EUM athletes are consuming just enough calories to maintain menstrual status, while their AMEN counterparts are not consuming enough.

CONCLUSION

Most research on obese and sedentary populations have shown that during periods of starvation and certain hypocaloric diets, there is a decreased peripheral conversion of T_4 to T_3 and an increased serum concentration of rT_3. It has been suggested that this decline in metabolically active thyroid hormones provides an important survival mechanism by slowing the rate of protein turnover and preserving an individual's lean body mass (Danforth et al., 1978). Nonetheless, the relationship between exercise and thyroid hormones in physically active menstruating women appears to be less conclusive. It has been suggested that mild thyroidal impairment may develop in physically active EUM women as decreases in both T_3 and rT_3 have been observed, with no change in T_4. Moreover, it has been hypothesized that the mechanism of these thyroid adaptations may be different in physically trained EUM women than during

times of caloric deprivation. One should not forget however, that studies examining the effects of chronic exercise on thyroid metabolism have lacked control of caloric intake and therefore may have confounded results.

The relationship between thyroid hormones (and LH pulsatility), diet and exercise has been examined more recently in menstruating, non-athletic women. Short-term studies indicate that energy intake or exercise *per se* does not trigger hormonal (ie. metabolic and reproductive) alterations. Findings indicate that it is the level of energy availability at which these changes occur. The physically active AMEN woman may be characterized by low caloric intake and low T_3 levels, in combination with high energy expenditures and may be below a necessary energy availability level to maintain reproductive function. Furthermore, varying degrees of reproductive dysfunction in this population have serious short- and long-term health consequences. For example, infertility, breast cancer, lipoprotein alternations and premature osteoporosis are all serious health ramifications associated with athletic menstrual irregularities. If in fact menstrual dysfunction is dependent on the level of energy availability, then these irregularities may be effectively prevented or reversed by dietary changes, without moderation in exercise regimen.

Thresholds examining the relationships between thyroid hormones and energy availabilities have been established for the non-athletic woman. Nonetheless, different thresholds may exist for the physically active AMEN and EUM woman, as these athletic populations appear to have similar caloric intakes to EUM sedentary women, despite their high level of energy expenditure. If in fact thresholds exist in the physically trained woman, it is important to establish these thresholds for each specific population (ie. EUM and AMEN), if healthful weight control recommendations are desired for the exercising woman.

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CHAPTER III

METHODS AND PROCEDURES

EXPERIMENTAL DESIGN (Table 3.1; Figure 3.1)

Subjects were quasi-randomly assigned to 5 groups of energy availability of 5.5, 11.4, 18.1, 25.0 and 37.4 kcal·kg FFM^{-1}·day^{-1}. Four of these groups (Groups 1 - 4) were designed to mimic the 4 groups created by Loucks and Heath (1994a), with Group 5 being created to examine the effects of an even lower energy availability on thyroid metabolism. The level of energy availability was determined by the number of calories consumed per day, minus the number of calories expended (through exercise) per day, in a laboratory on a stationary bike. The study began on the first through seventh day of each subject's menstrual cycle and involved 7-consecutive days of arriving at the Women's Health & Physical Activity Lab, between the hours of 5:30 A.M. and 10:30 A.M., at the University of Alberta in the Faculty of Physical Education & Recreation. At that time, a 10 ml fasting blood sample was taken from the antecubital vein by trained personnel, a urine sample was collected and body weight was determined. On days 3, 4, 5 and 6 of the experiment (ie. treatment days), each participant completed a supervised fixed volume of exercise equivalent to 30.0 kcal·kg FFM^{-1}·day^{-1} (depending on the subject, this ranged from 2 hr 24 min to 4 h 18 min of exercise each day). This exercise was performed in 30 minute bouts with 10 minutes of rest. In addition to the 4 days of exercise, subjects consumed a liquid nutritional supplement as their only food source (Ensure, Ross Laboratories, Columbus, Ohio) which was provided by lab personnel (ie. the liquid

nutritional supplement is explained under 'Treatment Variables - Dietary Intake'). Due to the amount of calories expended through exercise (ie. based on FFM) was the same for each participant, the amount of the liquid dietary product consumed determined each energy availability group.

Table 3.1 *Experimental Design of Energy Availability Groups*

Energy Availability Group Number	N (29)	Dietary Intake (kcal·kg FFM^{-1}·day^{-1})	Exercise Expenditure (kcal·kg FFM^{-1}·day^{-1})	Energy Availability (kcal·kg FFM^{-1}·day^{-1})
Group 1	5	69.1 ± 1.0 (70)	31.6 ± 0.3 (30)	37.4 ± 1.1 (40)
Group 2	6	54.8 ± 0.3 (55)	29.8 ± 1.0 (30)	25.0 ± 1.1 (25)
Group 3	6	49.2 ± 0.1 (49)	31.1 ± 0.7 (30)	18.1 ± 0.7 (19)
Group 4	6	41.2 ± 0.3 (41)	29.8 ± 0.5 (30)	11.4 ± 0.7 (11)
Group 5	6	34.9 ± 0.1 (35)	29.4 ± 0.7 (30)	5.5 ± 0.7 (5)

Values are means ± SE; Values in brackets are the exact values that were designated before treatment began

Each group had assigned caloric values (ie. based on FFM) for both dietary intake and exercise expenditure. However, allotment of the liquid nutritional supplement was rounded to the nearest 'Normal'

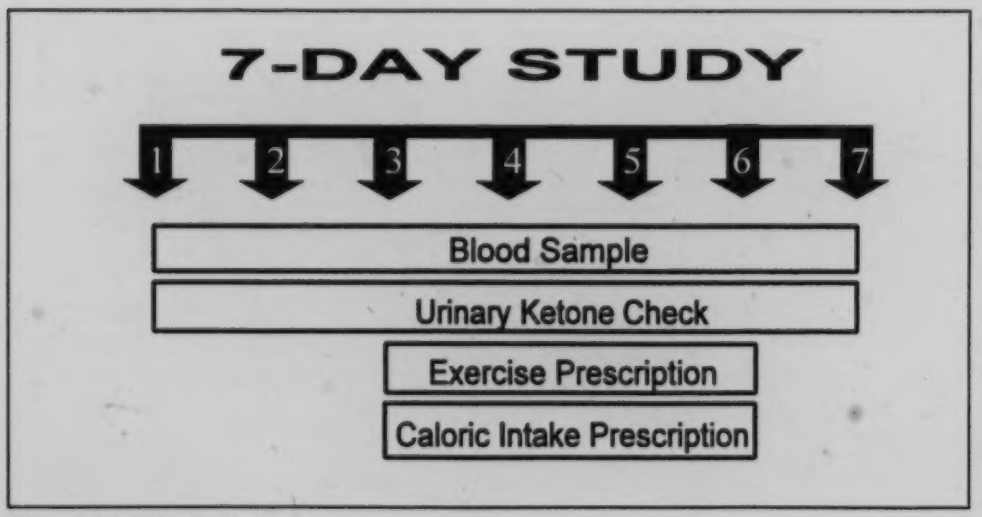

Figure 3.1 *7-Day Study*

½ can and the intensity of exercise was performed at ± 0.1 $L \cdot min^{-1}$ of oxygen, which were both causes for the minimal variation within groups.

BASELINE CHARACTERISTICS

I. Inclusion Criteria

Twenty-nine healthy, young, physically active, non smoking, women who completed the 7-day study, over 8 months from mid-September 1997 to the end of April 1998, were recruited from the University of Alberta and surrounding community. Admittance into the study required that the volunteers be: i) 17 to 35 years of age; ii) have an average menstrual cycle length for the previous 3 menstrual cycles of 24 to 37 days before starting the 7-day study; iii) free from use of oral contraceptives (for at least 3 months) or any other known medication to effect hormonal status; iv) no reported history of heart disease, liver disease, renal disease, diabetes or thyroid disorders; and v) have a $\dot{V}O_{2max} \geq 44.8$ $ml \cdot kg^{-1} \cdot min^{-1}$. Note, 1 subject withdrew from the 7-study due to personal reasons on Day 5 of 7.

This study received approval from the Ethics Committee in the Faculty of Physical Education & Recreation. Prior to participating in the study, subjects received a detailed explanation of study from the investigator and a written explanation of the study (ie. refer to Appendices 'Study Information Sheet'). Once each subject was satisfied with all study procedures, a consent form was signed by the subject, the investigator and a witness. Subjects also completed a 'Demographic Questionnaire' before participating in the study (refer to Appendices). This questionnaire surveyed each subject's use of medication, history of

disease, history of eating disorders, history of musculo-skeletal injuries, allergies, menstrual history and involvement (ie. mode, frequency, duration and intensity) in physical activities. All subjects were informed that they were free to withdraw from the study at any time, without prejudice.

Subjects were made aware of the risks associated with participating in the study. These risks included: 1) performing a $\dot{V}O_{2max}$ test could produce side effects such as fainting, muscle cramps or strain, lightheadedness, shortness of breath, nausea, abnormal heart beat or an abnormal blood pressure; 2) at the site of venipuncture, bruising or an infection could develop; 3) the presence or absence of the liquid nutritional supplement may cause bloating, a feeling of fullness, nausea, lightheadedness or hunger; and 4) throughout the 4 days of exercise on the stationary bike, muscle and groin soreness, and body fatigue could develop.

2. Aerobic Assessment

Before participating in the 7-day study, each subject's $\dot{V}O_{2max}$ was determined through the implementation of a bicycle ergometer test using standard open circuit spirometry (Horizon Metabolic Cart, Beckman Instruments Inc., Schiller Park, Illinois). Subjects started the test at a resistance of 1.5 kg, and pedaled at a frequency ranging from 50 to 80 RPM. Every 2 minutes, the resistance on the bike was increased 0.5 kg and the subjects were instructed to keep their RPM constant throughout the entire test. Once the subjects appeared to reach their VT, the resistance on the bike was then increased every minute until exhaustion. VT was identified when the subjects $\dot{V}E/\dot{V}CO_2$ (the ratio of the amount of air breathed to

the amount of CO_2 produced) reached a nadir prior to a sustained increase. Every minute and just prior to the resistance on the bike increasing, each subject's heart rate was determined using a heart rate monitor (Polar, Port Washington, New York). Two of the following three criteria were used to establish that $\dot{V}O_{2max}$ had been achieved: 1) a plateau in oxygen uptake (< 150 ml·kg^{-1}·min^{-1}) or peaking over in oxygen uptake with an increase in resistance; 2) an RER ≥ 1.1; and 3) within 5 beats of their age-predicated maximum heart rate.

3. Body Composition

Body composition was determined during the early follicular phase of the menstrual cycle, just prior to starting the 4 days of treatment (ie. liquid nutritional supplement and controlled exercise). Subjects were instructed to not eat anything 4 hours prior to the test (ie. to minimize food stuffs and gas production in the gastrointestinal tract) and to not perform exercise prior to the test that day. Height and weight were recorded before using the helium dilution technique to compute the residual lung volume (Motley, 1957). Once this testing procedure was completed, 9 skinfolds were then taken using Harpenden calipers. These skinfolds included: triceps, biceps, subscapular, midaxillary, iliac crest, abdominal, front thigh, rear thigh and medial calf (Certified Fitness Appraiser, 1993). From these skinfold measures, the sum of 4 skinfolds (triceps, biceps, subscapular and iliac crest) (Durnin & Womersley, 1974), the sum of 5 skinfolds (triceps, biceps, subscapular, iliac crest and medial calf) (Canadian Standardized Test of Fitness,

1986), and the sum of 6 skinfolds (triceps, subscapular, iliac crest, abdominal, rear thigh and front thigh) (Yuhasz, 1966) were determined.

The technique used for calculating FM, FFM and % body fat was determined by using body density. This was completed by weighing each subject underwater, correcting for water temperature and the trapped air in each subject (ie. residual lung volume and gastrointestinal tract volume), and calculating the percentage of body fat from body density using the formula of Brozek et al. (1963). This procedure was performed 6 to 10 times with the determined 'actual' residual lung volume (ie. calculated using the helium dilution technique) and 6 underwater weighings were completed using the 'predicted' residual volume (ie. determined using the woman's age and height) (Sensor Medics Corporation, 1998) for comparison.

For 4 subjects, it appeared as though using the 'actual' residual volume technique resulted in an over-estimation of % body fat (ie. by approximately 5 %). In such instances, where there appeared to be a discrepancy between the 'actual' determined % body fat (ie. using the hydrostatic technique) and skinfold data, the skinfold measures were used to predict % body fat using the Yuhasz (1966) formula. If the Yuhasz % body fat prediction was similar to the 'predicted' % body fat (and taking into consideration that the Yuhasz method underestimates % body fat) (Certified Fitness Appraiser, 1993), then the 'predicted' underwater weighing values were used instead of the 'actual' values, for future calculations (ie. determining the number of calories to consume and expend). The decision to use the 'predicted' body composition values was completed by experienced testers (the principal investigator and her supervisor).

4. Habitual 7-Day Dietary Intake

Each subject completed a 7-day diet record to calculate the mean caloric, CHO, fat and protein intake as an estimate of the subjects' habitual intake. Analysis was completed by using computer software (Food Processor, ESHA Research, Oregon). Recipes and nutrient information labels, when provided by participants, were entered into the computer program. Prior to completing the 7-day diet record, subjects completed a 3-day diet record, which was used for subjects to become familiar with the procedure, and to receive feedback on how they could improve the accuracy of reporting over the 7-days. The majority of the 7-day diet records were completed before the participants started the 7-day study, however, due to time restrictions, a few participants had to complete their 7-day diet record once completing the study. In these instances, subjects were given approximately 2 weeks before starting their 7-day diet record, in the hope that their dietary patterns had returned to normal, once the study had been completed.

TREATMENT VARIABLES

1. Group Assignment

Quasi-random assignment was completed when a subject began her first day of the 7-day study. Determination of each subject's assigned energy availability group was completed by picking one of the five groups (which had been written on a piece of paper) from a hat. This process was completed in this way, to ensure that subjects were assigned to each of the groups at approximately the same rate and to ensure

roughly equal distribution between the five groups, as the total number of participants to complete the study was not conclusive.

2. Start Date

Initially it was determined that subjects would have to start the study within the first six days of starting their menstrual cycle. However, two participants started the 7-day study on the seventh day of their menstrual cycle (this was the only option for them if they were to participate in the study). In addition, 2 subjects completed a 6-day study due to personal time restrictions. Therefore, for these 2 participants, only 2 days of baseline were used (eg. Day 1/6 and Day 2/6) instead of 3 days (eg. Day 1/7, 2/7 and 3/7).

3. Dietary Intake (4-day treatment)

The composition of the liquid nutritional supplement for each can of drink was as follows: 'Normal' can of Ensure: 250 kcal; 'Plus' can of Ensure: 355 kcal, with both types of drinks comprised of 55% CHO, 30% fat and 15% protein. Each subject was instructed to drink all of their Ensure provided to them before midnight, and to consume no other foods. There were no regulations of when the participants consumed their drink throughout the day. Subjects were permitted and encouraged to drink water *ad libitum*.

To track compliance with the study, a series of questions were verbally asked each morning. On study days 1, 2 and 3, these questions were asked:

i. Have you fasted since midnight ?

ii. Did you consume your usual amount of food and beverages yesterday ? (If no, please explain).

iii. Did you exercise yesterday ? (If yes, what did you do and is this a usual workout ? If not your usual workout, please explain)

The days prior to treatment, subjects were reminded to consume their usual amount of food and beverages and to perform their usual exercise routine. All subjects indicated that they consumed their usual amount of food and beverages and performed similar amounts of exercise to their usual routine.

On study days 4 through 7, these questions were asked:

i. Have you fasted since midnight ?

ii. Did you consume all of your liquid nutritional supplement yesterday ?

iii. Did you consume anything else besides your liquid nutritional supplement yesterday ?

iv. Did you exercise outside of the lab yesterday ? (If yes, what did you do ?)

All subjects indicated that they had consumed only water and their liquid nutritional supplement during the four days of treatment. Participants were reminded that it was very important that exercise energy expenditure (outside of the laboratory) be kept to a minimum and therefore some subjects were able to drive or take the bus to the University campus, instead of walking or riding their bike.

Some participants experienced difficulty consuming all of their liquid nutritional supplement, especially on their first day of treatment. It was recommended that subjects try to space their drinks out evenly throughout the day and to consume at least one drink while exercising. Since the drinks were thick, some participants found that diluting each drink with water assisted in enjoying the drink as much as possible. In addition, some participants froze their drinks (poured into a plastic cup), warmed the drink, and/or mixed various flavours together.

The labels from each can of drink were removed before the subjects were given their allotment because: (i) the brand of drink was to be kept confidential until the entire study was completed; (ii) there was concern that some individuals may not consume all of their drink, if they knew how many calories they were consuming; (iii) they may perform more exercise before or after exercising in the laboratory, if they knew how many calories they were consuming; (iv) negative psychological factors (eg. they may gain weight, they were not receiving enough calories to perform the exercise) may affect their continued participation in the study; (v) participants did not know that there were 'Normal' and 'Plus' cans, though some individuals were able to distinguish that there was a difference, due to the enhanced consistency of the 'Plus' drinks; and (vi) there was concern that if participants knew that they were consuming a small amount of calories per day, that they may be tempted to consume other sources of calories.

Subjects were given the choice of deciding which flavours that they would like to drink each day. The 'Normal' flavours were: strawberry, vanilla, chocolate, wildberry, butter pecan and orange cream. The 'Plus' flavours were: strawberry, vanilla and chocolate. However, subjects were only permitted to consume one 'dessert' flavour each day, these included: wildberry, butter pecan and orange cream. As there were no labels on each can of Ensure, and due to subjects frequently wanting to change the flavour of their drinks, a colour coding system was used to distinguish 'Normal' and 'Plus' cans. A red pen was used to indicate the flavour of any 'Plus' drink, and any colour other than red, was used to write the flavour of a 'Normal' drink.

In an attempt to minimize the range in the number of cans of drink each subject would receive (ie. 7 to 10 cans a day), subjects who had to consume a high amount of calories (Groups 1 & 2) were given

primarily 'Plus' cans (ie. to try to keep the number of cans they were having to consume to a minimum). Whereas, subjects who had to consume a low amount of calories (Groups 4 & 5), were primarily given 'Normal' cans. However, trying to keep within the range of 7 to 10 cans a day was not possible for everyone. Some participants consumed 6 cans of drink while others had to consume 11 cans of drink per day. Minimizing the range in the number of cans of drink was attempted as some participants who were in different groups completed the study at the same time and it was hoped that the psychological effects of seeing someone consume a lot more or a lot less calories did not affect them in a negative way. Note, ½ cans were assigned and consumed right after blood sampling, in an attempt to be as close as possible to the predetermined caloric intake for that individual.

Furthermore, individuals were instructed not to chew gum over the 4-days of treatment, due to its caloric content. As well, participants were permitted only to consume water and no other non-caloric beverages (eg. tea, coffee, diet soda pop) due to the effects that these beverages may have on their metabolic status. Unfortunately, some participants experienced symptoms that were most likely due to caffeine withdrawal (eg. headaches, shakiness). For this reason and for others with muscular pain, or menstrual cramps, individuals were allowed to take aspirin-like substances, in an effort to relieve discomfort. Subjects were also instructed to not consume any vitamins before giving a urine sample, as an excess of vitamin C could affect the ketone analysis (package insert, Bayer Inc., Etobicoke, Ontario).

4. Exercise (4-day treatment)

Each subject exercised at approximately 90% of their VT (which was determined from their $\dot{V}O_{2max}$ test). Total energy expenditure amounted to 30 kcal·kg FFM^{-1}·day^{-1} while riding on a Monark cycle ergometer, under continuous supervision in a sequence of 30 minute bouts interrupted by 10 minute rest periods. Throughout minutes 5 to 10 of each exercise bout, the subjects' oxygen uptake was measured directly using open circuit spirometry, ensuring the proper intensity ($\pm$ 0.1 L·min^{-1} of oxygen) was attained. If the intensity of exercise was not met, resistance and/or RPM were adjusted appropriately. Throughout the 30 minutes of exercise, heart rate, resistance and RPM were monitored and recorded every 5 minutes. To help pass the time during exercise, the subjects watched movies, listened to music, had friends and relatives come by (some even exercised with them), and the research lab assistants tried their best to keep conversation continuous and to impart enthusiasm.

From the metabolic indices recorded, oxygen consumption and RER values were averaged using the last 80 seconds of measurement. The RER values were then used to calculate the amount of work (ie. caloric expenditure) each subject had performed for that specific exercise bout. These values were then used to determine the total amount of calories expended (through exercise) for that treatment day.

BLOOD SAMPLING & ANALYSIS PROCEDURES

For 7 consecutive days, commencing 2 days before treatment, a 10 ml blood sample was taken and analyzed for T_4, T_3, fT_4 and fT_3. Estradiol and rT_3 blood samples were analyzed on 3 days (ie. experiment days 1, 3 and 7). Blood samples sat for 45 to 60 minutes at room temperature allowing to clot, and were

then centrifuged for 15 minutes at a speed of 3000 RPM. The serum was then pipetted and stored in a freezer (at -80°C) to be later assayed. E_2, T_4, T_3, fT_4 and fT_3 were assayed by means of radioimmunoassay (RIA) kits (Diagnostic Products Corporation, Los Angeles, California), as was rT_3 (BioChem ImmunoSystems Italia S.P.A., Casalecchio di Reno - (Bologna) Italia).

Standard curve concentrations were as follows: E_2 (0, 20, 50, 75, 150, 250, 500 $pg \cdot ml^{-1}$); T_4 (0, 1, 4, 8, 10, 16 $\mu g \cdot dL^{-1}$); T_3 (0, 20, 50, 75, 100, 200 $ng \cdot dL^{-1}$); fT_4 (0.0, 0.1, 0.54, 0.7, 1.4, 2.5 $ng \cdot dL^{-1}$); fT_3 (0.0, 0.5, 1.6, 3.7, 7.5, 11.0 $pg \cdot ml^{-1}$); and rT_3 (0.0, 0.025, 0.05, 0.10, 0.25, 0.50, 1.00, 2.00 $ng \cdot ml^{-1}$). Known concentrations for all hormones were used as controls in each assay and are as follows: E_2 (66.63, 198.57 $pg \cdot ml^{-1}$); T_4 (3.13, 8.31, 13.23 $\mu g \cdot dL^{-1}$); T_3 (89.12, 156.72 $ng \cdot dL^{-1}$); fT_4 (0.46, 1.36, 2.10 $ng \cdot dL^{-1}$); fT_3 (2.43, 4.18, 11.18 $pg \cdot ml^{-1}$); and rT_3 (0.49 $ng \cdot ml^{-1}$). All controls were obtained from Diagnostic Products Corporation (Los Angeles, California) with exception of rT_3, which were obtained from BioChem ImmunoSystems Italia S.P.A. (Casalecchio di Reno - (Bologna) Italia).

All hormone samples were measured as duplicates within a single assay according to the instructions provided by the manufacturers of the kits. While assaying for rT_3, technical problems occurred with the centrifuge during the analysis, and the time sequence for the 3 batches that had been prepared was disrupted. Controls for all 3 batches had been prepared and indicated that all batches had been treated in a similar fashion.

URINE SAMPLING & ANALYSIS PROCEDURES

A urine sample (minimum 10 ml) was collected on all 7-days of the study and assayed for the presence of acetoacetic acid with Keto-Diastix dipsticks (Bayer Inc., Etobicoke, Ontario). There was no control over whether or not participants had a morning void before collecting their urine sample. This test is based on the development of colours on the reagent area ranging from buff-pink for a negative reading, to purple when acetoacetic acid reacts with nitroprusside. After dipping the dipstick into the urine sample for 1 second, the dipstick was then tapped on the side of the urine bottle to rid of the excess urine and then held in a horizontal position for 30 seconds prior to determining the colour of the strip from the Keto-Diastix dipstick container label. Colour blocks on the bottle label were designated as 'Negative' (0.0 $mmol \cdot L^{-1}$), 'Trace' (0.5 $mmol \cdot L^{-1}$), 'Small' (1.5 $mmol \cdot L^{-1}$), 'Moderate' (4 $mmol \cdot L^{-1}$), or 'Large' (8-16 $mmol \cdot L^{-1}$).

STATISTICAL ANALYSIS

For each subject, T_4, T_3, fT_4 and fT_3 baseline concentrations were determined by averaging the serum concentrations measured on experiment days 1, 2 and 3. Reverse T_3 baseline concentrations were determined by averaging serum concentrations measured on experiment days 1 and 3. An estimate of each subject's response to treatment was determined by subtracting the same subject's thyroid hormone concentrations on experiment day 7 (ie. post-treatment) with that of the baseline estimate (ie. pre-treatment). However, for the 2 subjects who completed a 6-day study, they only had 2 days of baseline.

Therefore, for rT_3, in which only 2 days of baseline were determined for all subjects (ie. Day 1/7 and Day 3/7), Day 1/6 and Day 2/6 were used.

Each hormone was analyzed using a one-way analysis of variance (ANOVA) (ie. for change and % change) and a two-way ANOVA (ie. for group effect, main effect of time and an interaction effect). The Least Significant Difference (LSD) post-hoc test was used when appropriate, to determine which groups were statistically different from each other. HRs and RERs were analyzed by one-way, two-way and three-way ANOVA (ie. group vs day vs exercise bout). Due to the various number of exercise bouts that each subject performed on a daily basis, a standardized comparison was completed for HRs and RERs in which the first, third and last bout of exercise was examined. Significance was set at $P<0.05$. Individual Pvalues will be reported as recommended by Curran-Everett et al. (1998). When appropriate, the LSD post-hoc test was used to determine which group, day of treatment, and/or exercise bout, was statistically different from each other.

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CHAPTER IV

RESULTS

BASELINE CHARACTERISTICS

1. Subject Characteristics (Table 4.1)

Participants ranged in age from 17 to 35 years, with a mean age of 24.1 ± 0.9 years (mean ± standard error). There were no statistically significant differences among the groups for age. Subjects had previously participated in a wide variety of sports from the recreational to the international level. These sports included: aerobics, climbing (ie. wall, ice and mountain), cross-country skiing, cycling, extreme adventure racing, field hockey, in-line skating, mountain biking, resistance training, rowing, running, soccer, stair climbing, step aerobics, swimming, triathlon and ultimate frisbee.

2. Menstrual Status (Table 4.1)

Age of menarche ranged from 11 to 15 years, with a group mean age of menarche of 13.1 ± 0.2 years. Subjects gynecological ages were from 3 to 21 years, with a group mean gynecological age of 11.1 ± 0.9 years. When averaged, menstrual cycle length was 24.3 to 37.3 days (ie. the average of the 3 previous consecutive menstrual cycles prior to starting the study), with a group mean menstrual cycle length of 29.0 ± 0.6 days. There were no statistically significant differences among groups for age of menarche, gynecological age and the average of 3 menstrual cycles.

Table 4.1 ***Subject Characteristics, Menstrual Status, Aerobic Assessment, Body Composition***

Characteristic	All Groups	All Groups (Range)	Energy Availability Group					*P* Value
			Group 1	Group 2	Group 3	Group 4	Group 5	
Subject Characteristics								
Calendar age (yr)	24.1±0.9	17-35	26.0±1.6	24.7±2.2	22.7±2.3	21.7±0.8	26.0±2.8	0.484
Menstrual Status								
Age of Menarche (yr)	13.1±0.2	11-15	13.2±0.7	13.2±0.5	12.7±0.3	12.3±0.3	14.0±0.3	0.102
Gynecological age (yr)	11.1±0.9	3-21	12.8±2.1	11.5±2.1	10.0±2.3	9.3±0.8	12.0±2.8	0.784
x̄ Menstrual cycle length (days)	29.0±0.6	24.3-37.3	30.0±1.7	27.2±1.0	29.7±1.0	29.4±1.2	28.9±1.7	0.629
Aerobic Assessment								
$\dot{V}O_{2max}$ ($ml \cdot kg^{-1} \cdot min^{-1}$)	48.0±0.5	44.8-55.4	48.4±0.8	49.2±1.2	48.3±1.5	47.0±0.8	47.3±0.9	0.597
$\dot{V}O_{2max}$ ($L \cdot min^{-1}$)	2.95±0.1	2.26-3.88	2.8±0.1	3.1±0.2	3.0±0.2	3.0±0.2	2.9±0.1	0.767
Maximum heart rate ($beats \cdot min^{-1}$)	186.0±1.8	162-206	188.8±2.3	179.0±2.7	183.5±4.6	190.7±3.8	189.0±4.6	0.215
Ventilatory threshold ($ml \cdot kg^{-1} \cdot min^{-1}$)	29.4±1.0	20.4-42.4	29.0±1.8	30.7±1.8	28.6±3.2	31.5±2.0	26.9±1.9	0.620
Ventilatory threshold ($L \cdot min^{-1}$)	1.80±0.1	1.09-2.56	1.66±0.1	1.92±0.2	1.72±0.2	2.04±0.2	1.66±0.1	0.328
Ventilatory threshold (% of $\dot{V}O_{2max}$)	61.0±1.7	42.2-84.0	60.1±3.5	62.6±3.2	57.2±4.6	67.5±4.4	57.5±2.5	0.284
Body Composition								
Height (cm)	167.0±1.3	151.0-180.0	164.8±4.6	167.1±1.7	164.6±2.8	167.3±3.9	170.7±2.0	0.614
Weight (kg)	61.3±1.6	46.3-84.5	57.2±2.6	62.0±5.1	61.4±3.5	64.8±3.8	60.3±1.9	0.695
Fat mass (kg)	12.7±0.8	5.1-28.0	11.7±1.2	12.6±3.3	12.5±1.3	13.7±1.7	12.8±1.4	0.972
Fat free mass (kg)	48.6±1.0	36.5-62.7	45.6±2.5	49.5±2.1	48.9±2.5	51.2±2.8	47.4±1.6	0.544
Body fat (%)	20.3±0.9	11.0-33.0	20.5±2.0	19.0±3.2	20.2±1.4	20.9±1.8	21.2±2.1	0.960
4 skinfolds - Durnin & Womersley (mm)	40.5±2.6	18.7-79.5	36.8±4.1	40.0±7.4	39.1±4.2	44.7±7.8	41.5±6.3	0.930
5 skinfolds - CSTF (mm)	52.9±3.2	29.5-95.6	47.5±6.8	54.1±8.5	51.6±5.4	58.6±8.9	51.7±7.2	0.889
6 skinfolds - Yuhasz (mm)	99.6±4.1	63.8-152.3	92.8±7.5	97.9±12.4	98.6±8.0	106.6±10.5	100.7±8.8	0.907

Values are means ± SE. Differences between groups were considered statistically significant when $p<0.05$

3. Aerobic Assessment (Table 4.1)

$\dot{V}O_{2max}$, HR_{max} and VT were determined on a stationary Monark bike during a $\dot{V}O_{2max}$ test. Relative to body weight $\dot{V}O_{2max}$ ranged from 44.8 to 55.4 ml·kg^{-1}·min^{-1} and absolute $\dot{V}O_{2max}$ from 2.26 to 3.88 L·min^{-1}; with a group mean of 48.0 ± 0.5 ml·kg^{-1}·min^{-1} and 2.95 ± 0.1 L·min^{-1}, respectively. Maximum heart rates ranged from 162 to 206 beats·min^{-1}, with a group mean of 186.0 ± 1.8 beats·min^{-1}. Ventilatory thresholds expressed relative to body weight varied from 20.4 to 42.4 ml·kg^{-1}·min^{-1} and absolute values varied from 1.09 to 2.56 L·min^{-1}. When expressed as a percentage of their $\dot{V}O_{2max}$, VTs ranged from 42.2 to 84.0 %, with group mean values of 29.4 ± 1.0 ml·kg^{-1}·min^{-1}, 1.80 ± 0.1 L·min^{-1} and 61.0 ± 1.7 % of $\dot{V}O_{2max}$, respectively. There were no statistically significant differences among groups for $\dot{V}O_{2max}$ (ie. relative and absolute), maximum heart rate and ventilatory threshold (ie. relative, absolute and as a % of $\dot{V}O_{2max}$).

4. Body Composition (Table 4.1)

Subject height and weight ranged from 151.0 to 180.0 cm and 46.3 to 84.5 kg, respectively. Group means for height and weight were 167.0 ±1.3 cm and 61.3 ± 1.6 kg, respectively. Underwater weighing allowed for the determination of subjects FM, FFM and % body fat. Values ranged from 5.1 to 28.0 kg, 36.5 to 62.7 kg and 11.0 to 33.0 %, respectively. The group mean for FM was 12.7 ± 0.8 kg, for FFM was 48.6 ± 1.0 kg and for % of body fat was 20.3 ± 0.9 %. The sum of 4 skinfolds (Durnin & Womersley, 1974) ranged from 18.7 to 79.5 mm, with a group mean of 40.5 ± 2.6 mm. These values can be used to predict % body fat, which equate to an approximate range of 13 to 33 % and a group mean of 23 % (CFA, 1993). The sum of 5 skinfolds (CSTF, 1986) ranged from 29.5 to 95.6 mm, with a group mean of 52.9 ± 3.2 mm. These values can be used to determine percentiles and associated health risks. The sum of 5 skinfolds

consequently varied from approximately the 100th percentile (ie. in the upper health risk zone) to the 10th percentile (ie. in the lower health risk zone), with the group mean at the 65th percentile (ie. in the "healthy" zone) (CSTF, 1986). The sum of 6 skinfolds (Yuhasz, 1966) ranged from 63.8 to 152.3 mm, with a group mean of 99.6 $\pm$ 4.1 mm. These values can also be used to predict % body fat, which equate to a range of 9.4 to 28.6 % and a group mean of 17.1 % (CFA, 1993). These values are 1.6 to 4.4 % lower than the % body fat values determined when the underwater weighing technique was used. The CFA (1993) manual states that a limitation of the Yuhasz method is its under-prediction of % body fat compared to other methods. There were no statistically significant differences among groups for any of the body composition variables listed above.

5. Habitual 7-Day Dietary Intake

a) Caloric Intake (Table 4.2)

The number of calories consumed by all participants ranged from 1465 to 3190 kcal·day^{-1}. When expressed relative to FFM, caloric intake ranged from 29.3 to 68.8 kcal·kg FFM^{-1}·day^{-1}, with group means of 2295 $\pm$ 87.8 kcal·day^{-1} and 47.7 $\pm$ 2.0 kcal·kg FFM^{-1}·day^{-1}, respectively. There were no statistically significant differences among groups with respect to habitual caloric intake (ie. absolute and relative).

b) Carbohydrate Intake (Table 4.2)

The amount of CHO consumed by participants ranged from 203 to 509 grams·day^{-1}. When expressed relative to FFM, CHO intake ranged from 4.1 to 11.0 kcal·kg FFM^{-1}·day^{-1}, and expressed as a percentage of the total number of calories from 43.5 to 74.9 %. Group means were 346 $\pm$ 14.4 grams·day^{-1}, 7.1 $\pm$ 0.3 kcal·kg FFM^{-1}·day^{-1} and 60.5 $\pm$ 1.4 % of total calories, respectively. There were no statistically significant differences among groups.

c) Protein Intake (Table 4.2)

The amount of protein consumed by participants ranged from 49 to 123 grams·day^{-1}. When expressed relative to FFM, protein intake ranged from 1.1 to 2.8 kcal·kg FFM^{-1}·day^{-1}, and expressed as a percentage of the total number of calories from 11.4 to 18.0 %. Group means were 85 $\pm$ 3.7 grams·day^{-1}, 1.8 $\pm$ 0.1 kcal·kg FFM^{-1}·day^{-1} and 14.8 $\pm$ 0.3 % of total calories, respectively. There were no statistically significant differences among groups.

d) Fat Intake (Table 4.2)

The amount of fat consumed by participants ranged from 33 to 117 grams·day^{-1}. When expressed relative to FFM, fat intake ranged from 0.61 to 2.4 kcal·kg FFM^{-1}·day^{-1}, and expressed as a percentage of the total number of calories from 12.4 to 35.2 %. Group means were 62 $\pm$ 4.0 grams·day^{-1}, 1.3 $\pm$ 0.1 kcal·kg FFM^{-1}·day^{-1} and 24.3 $\pm$ 1.1 % of total calories, respectively. There were no statistically significant differences among groups.

Table 4.2 ***Habitual 7-day Dietary Intake***

Characteristic	All groups	All Groups (Range)	Energy Availability Group					*P* Value
			Group 1	Group 2	Group 3	Group 4	Group 5	
Caloric Intake								
kcal·day^{-1}	2295 ± 87.8	1465-3190	2455 ± 282.4	1994 ± 174.4	2407 ± 235.7	2320 ± 84.9	2326 ± 192.3	0.522
kcal·kg FFM^{-1}·day^{-1}	47.7 ± 2.0	29.3-68.8	53.6 ± 4.6	40.9 ± 4.4	50.2 ± 6.0	45.9 ± 2.7	48.9 ± 3.5	0.357
Carbohydrate Intake								
grams·day^{-1}	346 ± 14.4	203-509	371 ± 49.0	322 ± 33.4	356 ± 32.7	355 ± 25.9	330 ± 28.8	0.844
grams·kg FFM^{-1}·day^{-1}	7.1 ± 0.3	4.1-11.0	8.0 ± 0.9	6.5 ± 0.7	7.4 ± 0.7	7.0 ± 0.6	6.9 ± 0.4	0.643
% of total calories	60.5 ± 1.4	43.5-74.9	60.7 ± 5.6	64.8 ± 2.6	59.5 ± 2.7	60.9 ± 2.3	56.9 ± 2.8	0.529
Protein Intake								
grams·day^{-1}	85 ± 3.7	49-123	94 ± 10.1	71 ± 6.4	94 ± 9.9	88 ± 3.1	78 ± 9.0	0.202
grams·kg FFM^{-1}·day^{-1}	1.8 ± 0.1	1.1-2.8	2.1 ± 0.2	1.5 ± 0.2	2.0 ± 0.3	1.7 ± 0.1	1.7 ± 0.2	0.190
% of total calories	14.8 ± 0.3	11.4-18.0	15.4 ± 0.6	14.2 ± 0.4	15.6 ± 0.7	15.4 ± 0.6	13.3 ± 0.8	0.083
Fat Intake								
grams·day^{-1}	62 ± 4.0	33-117	62 ± 14.4	43 ± 2.9	69 ± 10.4	63 ± 3.3	74 ± 6.6	0.098
grams·kg FFM^{-1}·day^{-1}	1.3 ± 0.1	0.61-2.4	1.4 ± 0.3	0.9 ± 0.1	1.4 ± 0.3	1.3 ± 0.1	1.6 ± 0.1	0.097
% of total calories	24.3 ± 1.1	12.4-35.2	23.3 ± 4.2	19.5 ± 1.4	24.9 ± 2.1	24.7 ± 1.9	29.1 ± 1.3	0.073

Values are means ± SE. Differences between groups were considered statistically significant when $p < 0.05$

TREATMENT VARIABLES

1. Start Date (Table 4.3)

Subjects began the 7-day study on their second through seventh day of their menstrual cycle, with the group mean starting on the 3.9 ± 0.3 days. There were no statistically significant differences between groups starting the 7-day study in relation to the day of their menstrual cycle. Table 4.3 also indicates the average length of the 3 previous menstrual cycles. This allows for an estimate of where each group started and finished the study with respect to their menstrual cycle.

Table 4.3 ***Menstrual Cycle Length & Start Date***

Characteristic	All Groups	All Groups (Range)	Energy Availability Group 1	2	3	4	5	*P* Value
Start date of study (day of menstrual cycle)	3.9 ±0.3	2-7	4.2 ±0.6	4.5 ±0.8	4.2 ±0.7	3.0 ±0.8	3.5 ±0.7	0.594
$\bar{x}$ Menstrual cycle length (days)	29.0 ±0.6	24.3 - 37.3	30.0 ±1.7	27.2 ±1.0	29.7 ±1.0	29.4 ±1.2	28.9 ±1.7	0.629

Values are means ± SE. Differences between groups were considered statistically significant when $p<0.05$

2. Dietary Intake (4-day treatment)

a) Caloric Intake (Table 4.4; Figure 4.1)

The number of calories consumed by participants varied from 1500 (ie. subject in Group 5) to 3590 (ie. subject in Group 1) kcal·day^{-1}. Caloric intake for Group 1 and Group 5 were significantly different from all other groups as was Group 2 vs 4. The number of calories consumed by participants varied relative to their FFM from 34.6 (subject in Group 5) to 70.3 (subject in Group 1) kcal·kg FFM^{-1}·day^{-1}; statistically significant differences were found between all groups when comparing caloric intakes relative to FFM.

Table 4.4 ***Daily Dietary Intake and Energy Expended (through exercise) over 4-days of Treatment***

Characteristic	All Groups	All Groups (Range)	Energy Availability Group					*P* Value
			Group 1	Group 2	Group 3	Group 4	Group 5	
Dietary Intake (4-day treatment)								
kcal·day^{-1}	2381 ±107.5	1500-3590	3156 ± 209.9	2712 ± 118.6	2406 ± 117.9	2106 ± 99.5	1657 ± 58.0	0.000[a]
kcal·kg FFM^{-1}·day^{-1}	49.2 ± 2.2	34.6-70.3	69.1 ± 1.0	54.8 ± 0.3	49.2 ± 0.1	41.2 ± 0.3	34.9 ± 0.1	0.000[b]
Difference between 4-day Treatment Diet & 7-day Habitual Dietary Intake								
kcal·day^{-1}	86 ± 133.8	-1266-1270	701 ± 209.9	718 ± 226.1	-2 ± 290.3	-214 ± 124.9	-669±166.6	0.000[c]
kcal·kg FFM^{-1}·day^{-1}	1.5 ± 2.8	-27.3-28.5	15.5 ± 4.6	13.9 ± 4.4	-0.9 ± 6.0	-4.7 ± 2.5	-14.0 ± 3.5	0.000[d]
Exercise (4-day treatment)								
$\dot{V}O_2$ (L·min^{-1})	1.61 ± 0.1	1.01-2.15	1.50 ± 0.1	1.65 ± 0.1	1.54 ± 0.2	1.81 ± 0.1	1.52 ± 0.1	0.28
% $\dot{V}O_{2max}$	54.5 ± 1.2	38.9-66.2	54.0 ± 1.5	54.2 ± 2.6	51.4 ± 3.4	60.0 ± 2.2	53.0 ± 2.2	0.177
HR (beats·min^{-1})	135 ± 2.7	110-163	135 ± 8.0	131 ± 5.6	133 ± 5.9	146 ± 6.4	132 ± 4.2	0.424
% HR_{max}	73.1 ± 2.7	65.9-84.4	74.4 ± 15.1	73.2 ± 2.1	72.0 ± 1.7	76.4 ± 2.8	70.0 ± 1.7	0.361
Duration (min·day^{-1})	185.4 ± 4.7	144-258	182.4 ± 8.4	182.5 ± 7.5	200.3 ± 15.3	170.5 ± 9.4	190.7 ± 8.0	0.337
Exercise Energy Expenditure (4-day treatment)								
kcal·day^{-1}	1473 ± 34.1	1162-1910	1441 ± 82.0	1474 ± 68.6	1517 ± 71.6	1529 ± 100.1	1397 ± 69.7	0.746
kcal·kg FFM^{-1}·day^{-1}	30.3 ± 0.3	26.9-33.5	31.6 ± 0.3	29.8 ± 1.0	31.1 ± 0.7	29.8 ± 0.5	29.4 ± 0.7	0.149
RER	0.90 ± 0.0	0.86-0.94	0.93 ± 0.0	0.91 ± 0.0	0.90 ± 0.0	0.89 ± 0.0	0.88 ± 0.0	0.000[e,g]
Energy Availability (the difference between 4-day dietary intake & 4-day exercise energy expenditure)								
kcal·day^{-1}	909 ± 98.0	141-1978	1715 ± 130.4	1238 ±84.0	889 ± 62.6	576 ± 21.3	260 ± 28.3	0.000[h]
kcal·kg FFM^{-1}·day^{-1}	18.9 ± 2.1	2.9-39.3	37.4 ± 1.1	25.0 ± 1.1	18.1 ± 0.7	11.4 ± 0.7	5.5 ± 0.7	0.000[i]
Body Weight Changes (from baseline to post-treatment)[k]								
$\bar{x}$ Days 1,2,3 - Day 7(kg)	-1.3 ± 0.2	-4.7-0.2	-0.5 ± 0.3	-0.8 ± 0.2	-0.8 ± 0.3	-2.6 ± 0.5	-1.8 ± 0.3	0.001[l]
$\bar{x}$ Days 1,2,3 - Day 7(%)	-2.1 ± 0.3	-5.7-0.3	-1.0 ± 0.3	-1.2 ± 0.3	-1.2 ± 0.4	-3.9 ± 0.5	-2.9 ± 0.5	0.001[m]

Values are means ± SE. Differences between groups were considered statistically significant when $p < 0.05$
a, b, c, d, e, f, g, h, i, j, k, l, m = see Table 4.5 for differences between groups

Table 4.5 ***Statistically Significant Group Differences for Table 4.4***

Statistically Significant Effect	Characteristic	Figure	Significant Differences between Energy Availability Groups (Post-Hoc: LSD Test)	
a	Dietary Intake ($kcal \cdot day^{-1}$)	-	1 vs ALL; 2 vs 4; 5 vs ALL	
b	Dietary Intake ($kcal \cdot kg\ FFM^{-1} \cdot day^{-1}$)	4.1	ALL vs ALL	
c	Δ Between 4-day Treatment Diet & 7-day Habitual Dietary Intake ($kcal \cdot day^{-1}$)	-	1 vs 3; 1 vs 4; 1 vs 5; 2 vs 3; 2 vs 4; 2 vs 5; 3 vs 5	
d	Δ Between 4-day Treatment Diet & 7-day Habitual Dietary Intake ($kcal \cdot kg\ FFM^{-1} \cdot day^{-1}$)	4.2	1 vs 3; 1 vs 4; 1 vs 5; 2 vs 3; 2 vs 4; 2 vs 5; 3vs 5	
e	Exercise (4-day treatment): Heart Rate (All Groups; Day vs Exercise Bout)	4.4	Interaction effect between Day and Exercise Bout ($p = 0.000$) See Table 4.6	
f	Exercise (4-day treatment): RER (5 Groups)	4.6	1 vs ALL; 2 vs 4; 2 vs 5; 3 vs 5	
g	Exercise Energy Expenditure (4-day treatment): RER (Day vs Exercise Bout)	4.7	Interaction effect between Day and Exercise Bout ($p = 0.000$) See Table 4.7	
h	Energy Availability ($kcal \cdot day^{-1}$)	-	ALL vs ALL	
i	Energy Availability ($kcal \cdot kg\ FFM^{-1} \cdot day^{-1}$)	4.9	ALL vs ALL	
j	Body Weight Changes (from baseline to post-treatment)	-	Main effect of time from baseline to post-treatment ($p = 0.000$)	
k	Body Weight Changes (from baseline to post-treatment)	4.1	Interaction effect between groups from baseline to post-treatment ($p = 0.001$)	
			Baseline All groups different except 3 vs 5	Post-Treatment ALL vs ALL
l	Body Weight Changes (kg)	-	1, 2, 3 vs 4, 5	
m	Body Weight Changes (%)	4.11	1, 2, 3 vs 4, 5	

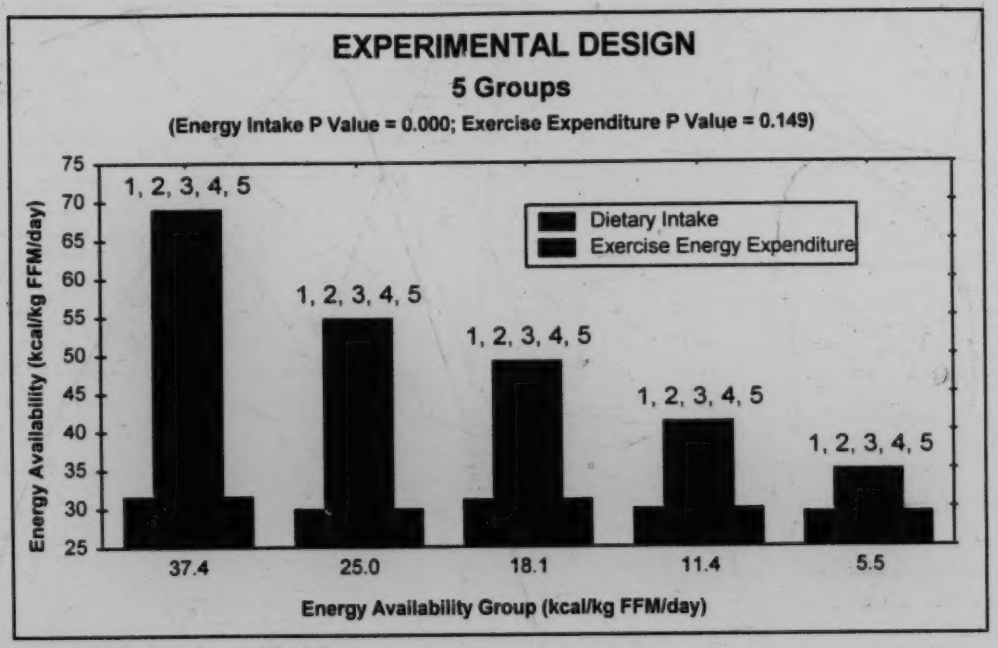

Figure 4.1 *Experimental Design*
Matching numbers above means indicate which groups were different from each other ($P<0.05$)

b) Difference between 4-day & 7-day Caloric Intakes (Table 4.4; Figure 4.2)

Caloric differences emerged between the 4-day assigned diet and 7-day habitual diet ranging from a deficit of (-) 1266 (subject in Group 5) to a surplus of 1270 (subject in Group 1) kcal·day^{-1}. Statistically significant differences were found between groups 1 vs 3; 1 vs 4; 1 vs 5; 2 vs 3; 2 vs 4; 2 vs 5 and 3 vs5. The number of calories consumed by participants varied with respect to FFM from a deficit of (-) 27.3 (subject in Group 5) to a surplus of 28.5 (subject in Group 1) kcal·kg FFM^{-1}·day^{-1}. Statistically significant differences were found between groups 1 vs 3; 1 vs 4; 1 vs 5; 2 vs 3; 2 vs 4; 2 vs 5 and 3 vs 5.

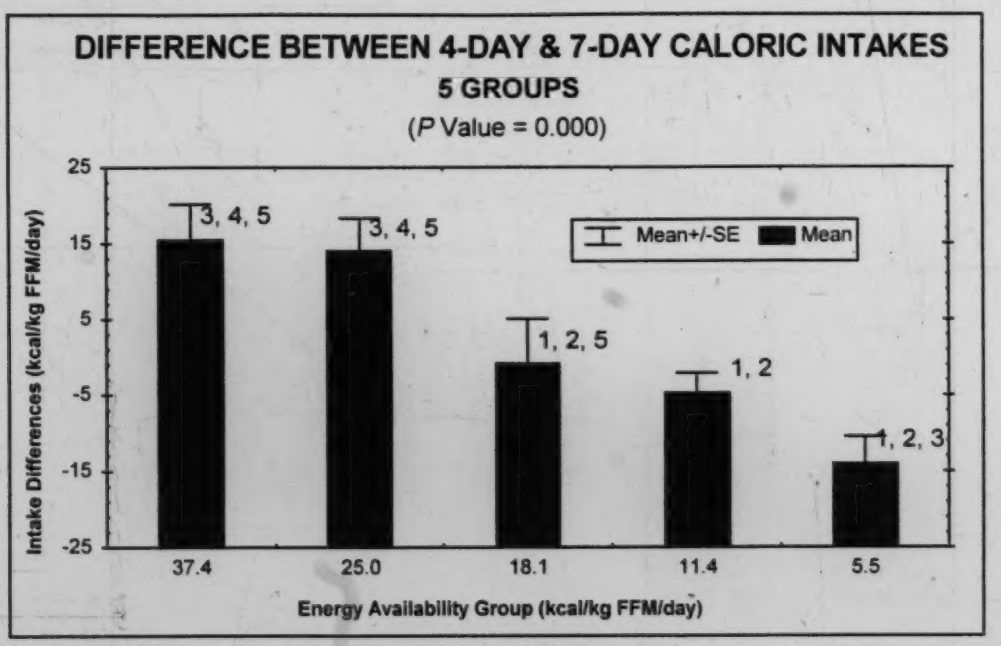

Figure 4.2 *Difference between 4-day & 7-day Caloric Intakes*
Matching numbers above means indicate which groups were different from each other ($P<0.05$)

3. Exercise (4-day treatment)

a) Intensity (Table 4.4 & 4.6 ; Figures 4.3 - 4.5)

The intensity at which each subject exercised for over 4-days ranged from 1.01 to 2.15 L·min^{-1} and from 38.9 to 66.2 % $\dot{V}O_{2max}$, with group means of 1.61 ± 0.1 L·min^{-1} and 54.5 ± 1.2 % $\dot{V}O_{2max}$, respectively. Heart rates were collected every 5 minutes during exercise and averaged over the 4-days of exercise. Four-day average heart rates ranged from 110 to 163 beats·min^{-1} and from 65.9 to 84.4 % HR_{max}, with group means of 135 ± 2.7 beats·min^{-1} and 73.1 ± 2.7 % HR_{max}, respectively. There were no statistically significant differences between groups with respect to the intensity (i.e. oxygen consumption and the average of 4-days of HRs) of exercise during treatment. However, when examining HRs over each

day of the 4-days of exercise, statistically significant differences were found. Statistical results indicated that the HRs on the 4th day of exercise, were statistically lower than all other days. In addition, HRs for exercise bout 1, were statistically higher compared to the HRs during the third and last exercise bout. Furthermore, a 2-way ANOVA indicated that there was an interaction between the day of exercise and the exercise bout. Numerous interactions were found between day of exercise and exercise bout, however, the majority of interactions were accounted for by the first exercise bout during days 1, 2 and 3 of exercise compared to all other combinations of day of exercise and exercise bout. Another interesting observation when examining HRs over each day of exercise, is that it appears as though Group 4 was exercising at higher HRs, however, no statistically significant differences were found between groups.

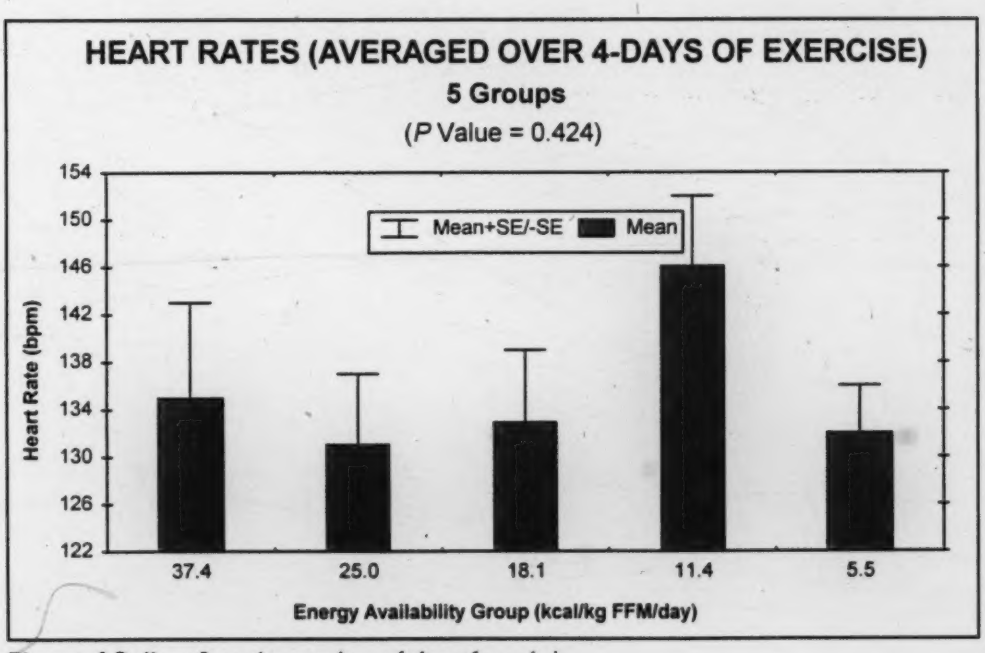

Figure 4.3 *Heart Rates (averaged over 4-days of exercise)*

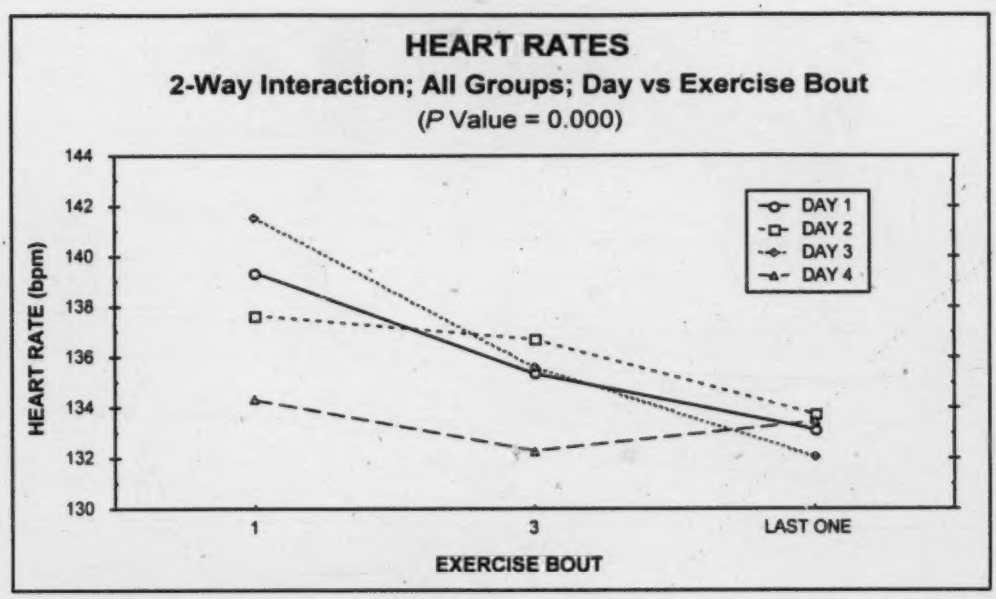

Figure 4.4 *Heart Rates: Day vs Exercise Bout*

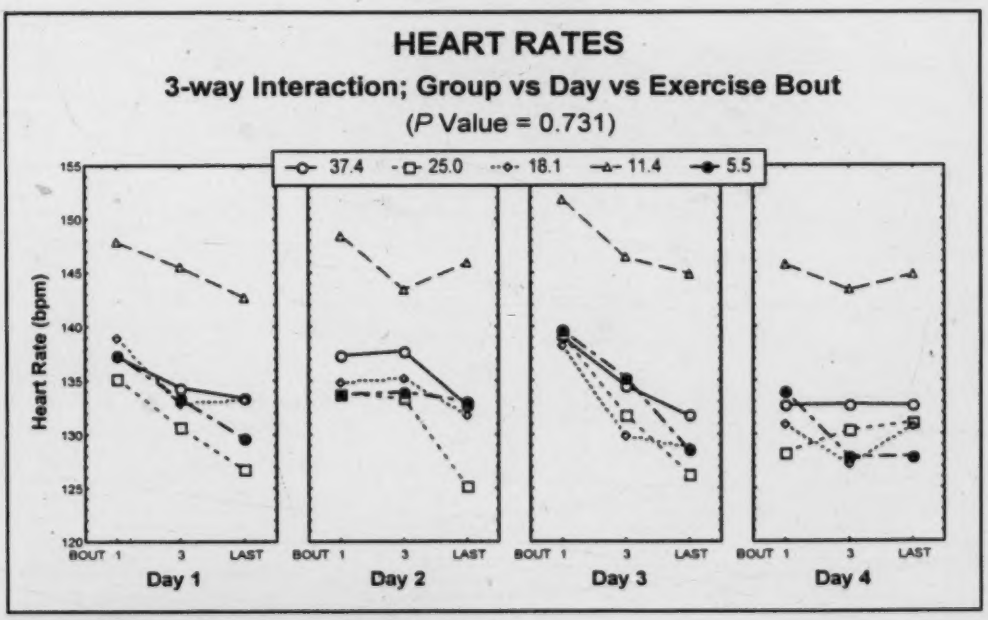

Figure 4.5 *Heart Rates: Group vs Day vs Exercise Bout*
Inset defines energy availability group assignment (kcal·kg FFM^{-1}·day^{-1})

Table 4.6 ***HR: Interaction Effect - Day (1, 2, 3 & 4) vs Exercise Bout (1st, 3rd & last one)***

	1st Bout Day 1	1st Bout Day 2	1st Bout Day 3	1st Bout Day 4	3rd Bout Day 1	3rd Bout Day 2	3rd Bout Day 3	3rd Bout Day 4	Last Bout Day 1	Last Bout Day 2	Last Bout Day 3	Last Bout Day 4
1st Bout Day 1	1st Bout Day 1			✔	✔	✔	✔	✔	✔	✔	✔	✔
1st Bout Day 2		1st Bout Day 2	✔	✔				✔	✔	✔	✔	✔
1st Bout Day 3		✔	1st Bout Day 3	✔	✔	✔	✔	✔	✔	✔	✔	✔
1st Bout Day 4	✔	✔	✔	1st Bout Day 4								
3rd Bout Day 1	✔		✔		3rd Bout Day 1			✔	✔	✔	✔	
3rd Bout Day 2	✔		✔			3rd Bout Day 2		✔			✔	✔
3rd Bout Day 3	✔		✔				3rd Bout Day 3	✔			✔	
3rd Bout Day 4	✔	✔	✔		✔	✔	✔	3rd Bout Day 4				
Last Bout Day 1	✔	✔	✔			✔			Last Bout Day 1			
Last Bout Day 2	✔	✔	✔			✔				Last Bout Day 2		
Last Bout Day 3	✔	✔	✔		✔	✔	✔				Last Bout Day 3	
Last Bout Day 4	✔	✔	✔			✔						Last Bout Day 4

✔ = statistically significant interaction effect ($P > 0.05$)

b) Duration (Table 4.4)

The amount of time each participant exercised ranged from 144 to 258 min·day^{-1} (i.e. 2 hr 24 min to 4 hr 18 min), with a group mean of 185.4 ± 4.7 min·day^{-1}. This range approximates completing 5 exercise bouts (of 30 min each) to 8.5 exercise bouts. There were no statistically significant differences between groups with respect to the duration of exercise during the 4 days of treatment.

4. Exercise Energy Expenditure (4-day treatment)

a) Energy Expenditure (Table 4.4; Figure 4.1)

The number of calories subjects expended on the stationary bike ranged from 1162 to 1910 kcal·day^{-1}, with a group mean of 1473 ± 34.1 kcal·day^{-1}. Expressed relative to FFM, the amount of energy expended ranged from 26.9 to 33.5 kcal·kg FFM^{-1}·day^{-1}, with a group mean of 30.3 ± 0.3 kcal·kg FFM^{-1}·day^{-1}. By experimental design, each subject should have expended approximately 30 kcal·kg FFM^{-1}·day^{-1}. There were no statistically significant differences between groups.

b) RER (Table 4.4 & 4.7; Figures 4.6 - 4.8)

RERs were recorded every exercise bout after approximately 5 min of cycling for 5 to 10 minutes. Average 4-day RER values ranged from 0.860 (subject in Group 5) to 0.940 (subject in Group 1), with a group mean of 0.899 ± 0.00. Statistically significant differences were found between the 4-day RER values: 1 vs ALL; 2 vs 4; 2 vs 5 and 3 vs 5. When examining all days together, a statistical decrease in RER

values occurred from the first to the third to the last exercise bout. In addition, RERs for the first day of exercise, were statistically higher than all other days. Furthermore, 2-way ANOVA analysis indicated that there was an interaction between the day of exercise and the exercise bout.

For each exercise day, RER values showed a decreasing trend from exercise bouts 1, 3 and the subjects' last bout. However, no statistically significant differences were found in RER values between the 5 groups, for each day of exercise. Although Group 1's RER values were predominantly higher and Group 5's RER values were mostly at the lower end of the range.

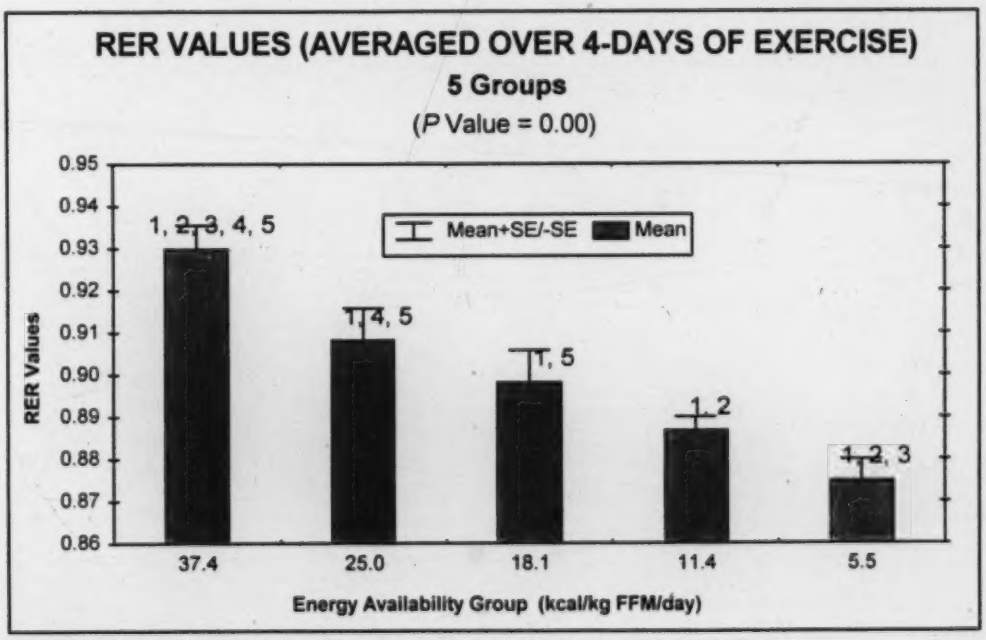

Figure 4.6 *RER Values (averaged over 4-days of exercise)*
Matching numbers above means indicate which groups were different from each other ($P<0.05$)

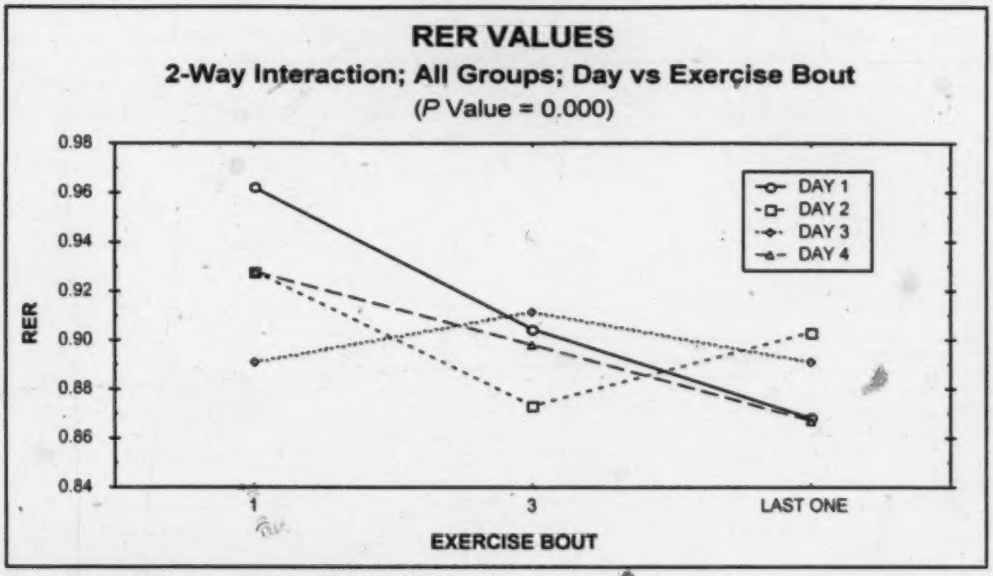

Figure 4.7 *RER Values: Day vs Exercise Bout*

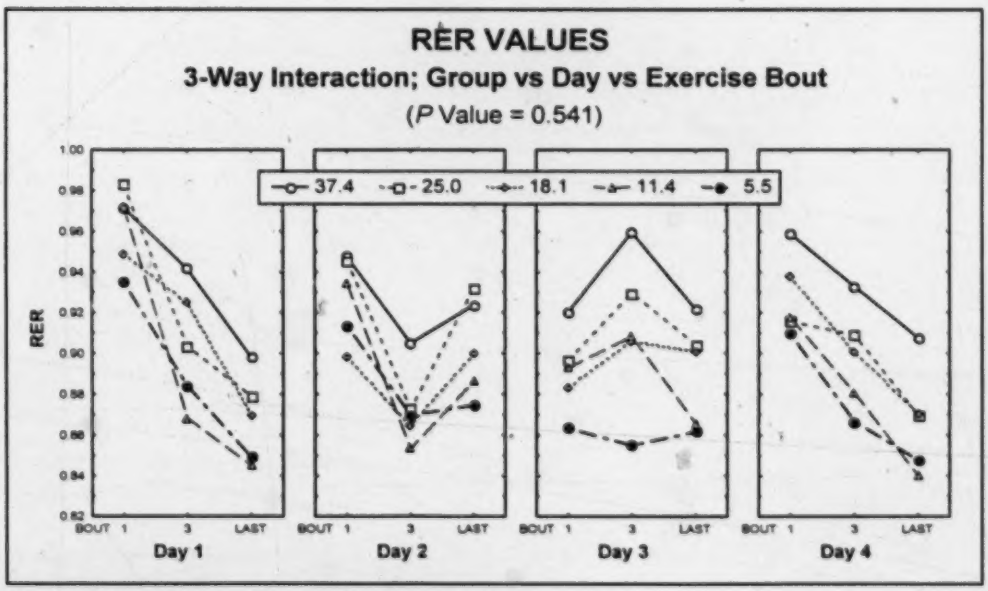

Figure 4.8 *RER Values: Group vs Day vs Exercise Bout*
Inset defines energy availability group assignment (kcal·kg FFM $^{-1}$·day $^{-1}$)

Table 4.7 ***RER: Interaction Effect - Day (I, 2, 3 & 4) vs Exercise Bout (Ist, 3rd & last one)***

	Ist Bout Day I	Ist Bout Day 2	Ist Bout Day 3	Ist Bout Day 4	3rd Bout Day I	3rd Bout Day 2	3rd Bout Day 3	3rd Bout Day 4	Last Bout Day I	Last Bout Day 2	Last Bout Day 3	Last Bout Day 4
Ist Bout Day I	Ist Bout Day I	✔	✔	✔	✔	✔	✔	✔	✔	✔	✔	✔
Ist Bout Day 2	✔	Ist Bout Day 2	✔		✔	✔	✔	✔	✔	✔	✔	✔
Ist Bout Day 3	✔	✔	Ist Bout Day 3	✔		✔	✔		✔			✔
Ist Bout Day 4	✔		✔	Ist Bout Day 4	✔	✔	✔	✔	✔	✔	✔	✔
3rd Bout Day I	✔	✔		✔	3rd Bout Day I	✔			✔			✔
3rd Bout Day 2	✔	✔	✔	✔	✔	3rd Bout Day 2	✔	✔		✔	✔	
3rd Bout Day 3	✔	✔	✔	✔		✔	3rd Bout Day 3		✔		✔	✔
3rd Bout Day 4	✔	✔		✔		✔		3rd Bout Day 4	✔			✔
Last Bout Day I	✔	✔	✔	✔	✔		✔	✔	Last Bout Day I	✔	✔	
Last Bout Day 2	✔	✔		✔		✔			✔	Last Bout Day 2		✔
Last Bout Day 3	✔	✔		✔		✔	✔		✔		Last Bout Day 3	✔
Last Bout Day 4	✔	✔	✔	✔	✔		✔	✔		✔	✔	Last Bout Day 4

✔ = statistically significant interaction effect ($P > 0.05$)

5. Energy Availability (Table 4.4; Figure 4.9)

Energy availability was set for each participant. Energy availability varied from 141 (subject in Group 5) to 1978 (subject in Group 1) kcal·day^{-1}. Energy availability expressed relative to FFM, ranged from 2.9 (subject in Group 5) to 39.3 (subject in Group 1) kcal·kg FFM^{-1}·day^{-1}. Statistically significant differences were found between all groups, for both kcal·day^{-1} and kcal·kg FFM^{-1}·day^{-1}.

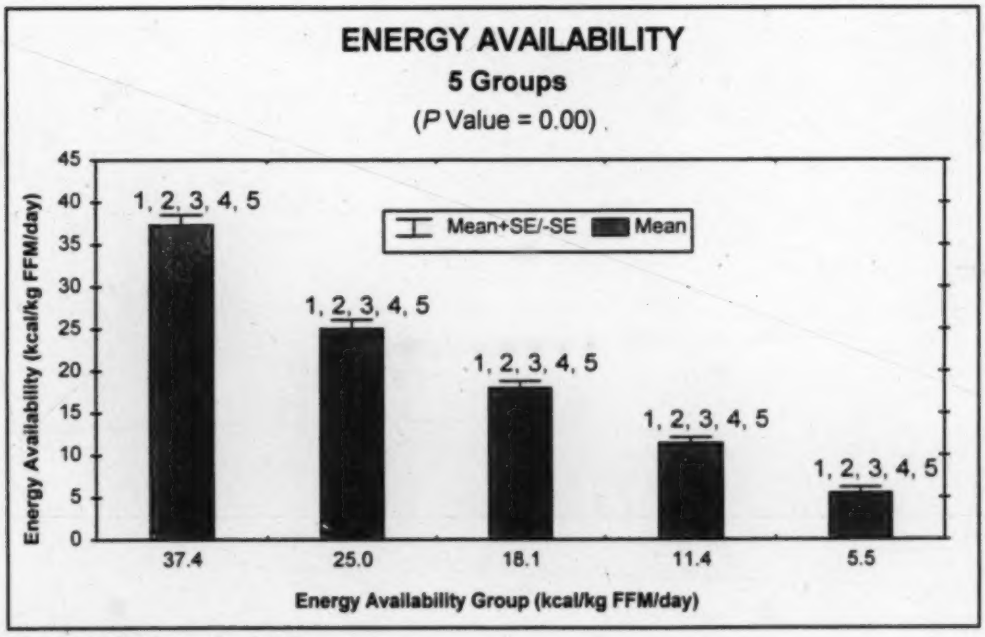

Figure 4.9 *Energy Availability*
Matching numbers above means indicate which groups were different from each other ($P<0.05$)

6. Body Weight Changes (Table 4.4; Figure 4.10 - 4.11)

Subjects change in body weight ranged from gaining (+) 0.2 kg (subject in Group 1) to losing (-) 4.7 kg (subject in Group 4). Statistically significant differences were found in the change of body weight between groups 1, 2, 3 vs 4, 5. Furthermore, a statistically significant main effect of time from baseline (average of days 1, 2 and 3) to post-treatment (morning of day 7) was found for the entire group. An interaction effect occurred, indicating that all groups were different from each other at baseline except for groups 3 vs 5, and at post-treatment all groups were different from each other. Percent change in body weight ranged from gaining (+) 0.3 % (subject in Group 1) to losing (-) 5.7 % (subject in Group 4) of body weight. Statistically significant differences were found in the % change of body weight between groups 1, 2, 3 vs 4, 5.

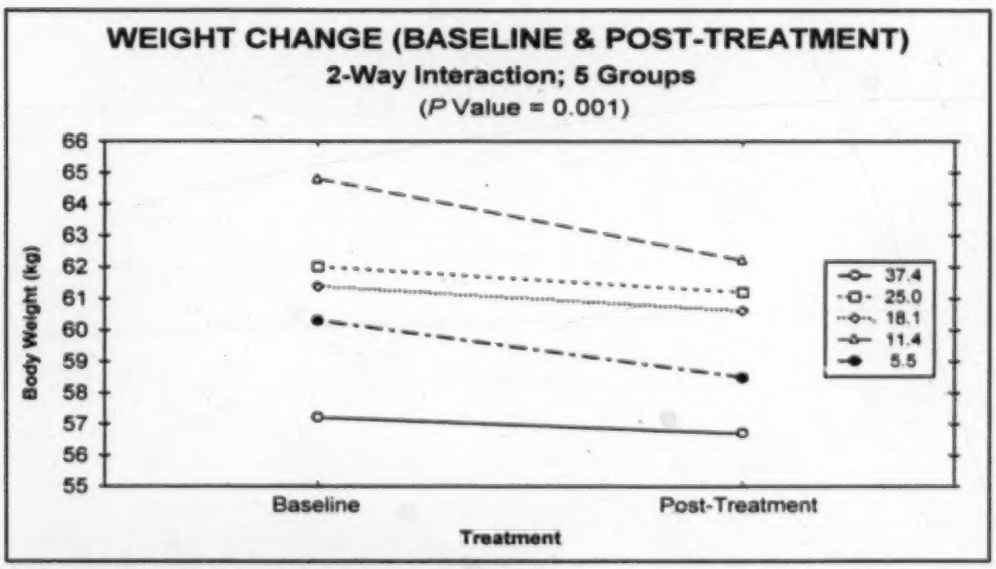

Figure 4.10 *Weight Change (baseline & post-treatment)*
Inset defines energy availability group assignment (kcal·kg FFM^{-1}·day^{-1})

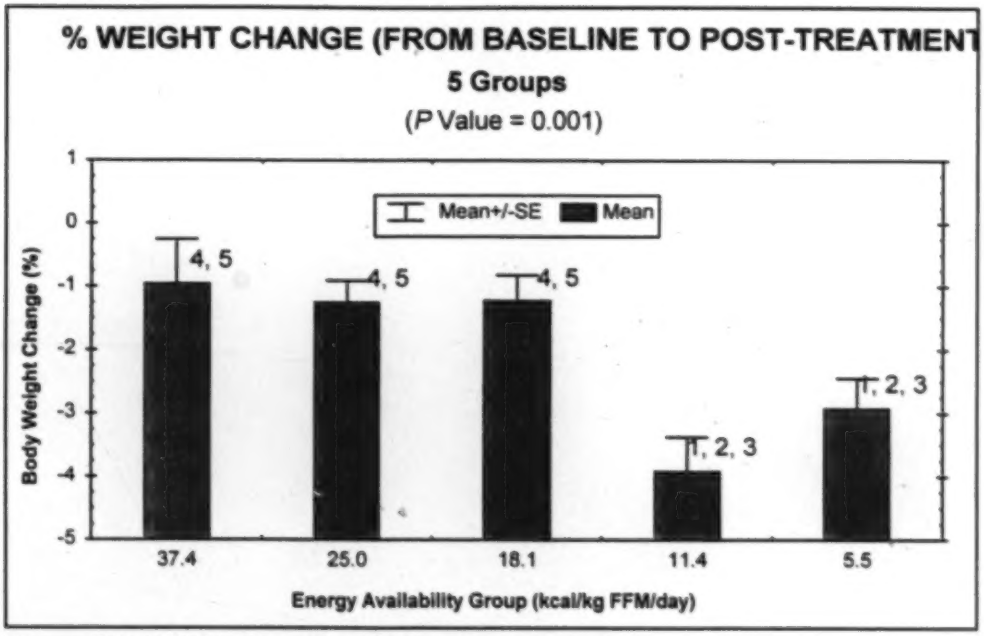

Figure 4.II *% Weight Change (from baseline to post-treatment)*
Matching numbers above means indicate which groups were different from each other ($P<0.05$)

7. Ketone (Acetoacetic Acid) Analysis

The level of acetoacetic acid (if any) in the subjects urine was determined each morning. Five positive results were obtained throughout the study. Of these 5 positive results, 4 were 'Trace' (0.5 $mmol \cdot L^{-1}$) amounts. The group and the day of the study of each positive result were as follows: 1 subject in Group 1, Day 6/7; 2 subjects in Group 2, Day 5/7 & Day 7/7; and 1 subject in Group 5 on Days 5/7 & 7/7. The subject in Group 5 , had a 'Trace' amount on Day 5/7 and on Day 7/7 she had a 'Moderate' (4.0 $mmol \cdot L^{-1}$) amount of acetoacetic acid in her urine.

HORMONES

1. Intra-Assay Performance

The intra-assay performance for all analyzed hormones was determined. The coefficient of variation (CV) was calculated for each set of duplicate tubes for each subject, and then a random sample of 5 % of the total number of tubes were used to calculate the mean CV. The intra-assay mean CV for the following hormones were: E_2 (5.0 %); T_4 (4.6 %); T_3 (4.5 %); fT_4 (6.4 %); fT_3 (8.5 %); and rT_3 (8.9 %).

2. Estradiol (Table 4.8; Figures 4.12)

Baseline values on Day 1 ranged from 23.4 to 97.2 $pg \cdot ml^{-1}$, with a group mean of 47.2 $\pm$ 3.1 $pg \cdot ml^{-1}$ and on Day 3 from 33.6 to 91.1 $pg \cdot ml^{-1}$, with a group mean of 52.4 $\pm$ 3.1 $pg \cdot ml^{-1}$. Post-treatment values on Day 7 ranged from 29.3 to 269.0 $pg \cdot ml^{-1}$, with a group mean of 77.9 $\pm$ 9.9 $pg \cdot ml^{-1}$. It should be noted that 3 subjects had E_2 concentrations greater than 150 $pg \cdot ml^{-1}$ on Day 7. Their respective groups were as follows: 2 women in Group 2 (189.8 and 269.0 $pg \cdot ml^{-1}$) and 1 woman in Group 4 (150.2 $pg \cdot ml^{-1}$). In addition to these differences, a statistically significant main effect of time occurred between Day 1 vs Day 7 and Day 3 vs Day 7, with no statistically significant interaction effect.

Table 4.8 *Estradiol Concentrations (pg·ml^{-1})*

Energy Availability Group (kcal·kg FFM^{-1}·day^{-1})	Baseline Day 1	Baseline Day 3	Post-Treatment Day 7	Start date of study (Day of menstrual cycle)	x̄ length of menstrual cycle (Days)
Group 1: 37.4	47.2 ± 8.9	59.8 ± 10.3	73.4 ± 10.9	4.2 ± 0.6	30.0 ± 1.7
Group 2: 25.0	61.8 ± 8.1	67.1 ± 8.2	133.0 ± 34.2	4.5 ± 0.8	27.2 ± 1.0
Group 3: 18.1	43.5 ± 4.8	49.7 ± 2.9	60.4 ± 6.5	4.2 ± 0.7	29.7 ± 1.0
Group 4: 11.4	37.6 ± 3.3	43.5 ± 3.1	58.4 ± 18.6	3.0 ± 0.8	29.4 ± 1.2
Group 5: 5.5	46.1 ± 6.3	43.3 ± 3.4	63.3 ± 14.6	3.5 ± 0.7	28.9 ± 1.7
All Groups	47.2 ± 3.1	52.4 ± 3.1	77.9 ± 9.9[a]	3.9 ± 0.3	29.0 ± 0.6
All Groups (Range)	23.4-97.2	33.6-91.1	29.3-269.0	2-7	24.3-37.3
P Value				0.594	0.629

Values are means ± SE. Differences between groups were considered statistically significant when $p<0.05$
a = statistically significant main effect of time; Day 1 vs Day 7 & Day 3 vs Day 7 ($p = 0.000$)
No statistically significant main interaction effect between groups for Days 1, 3 and 7 ($p = 0.204$)

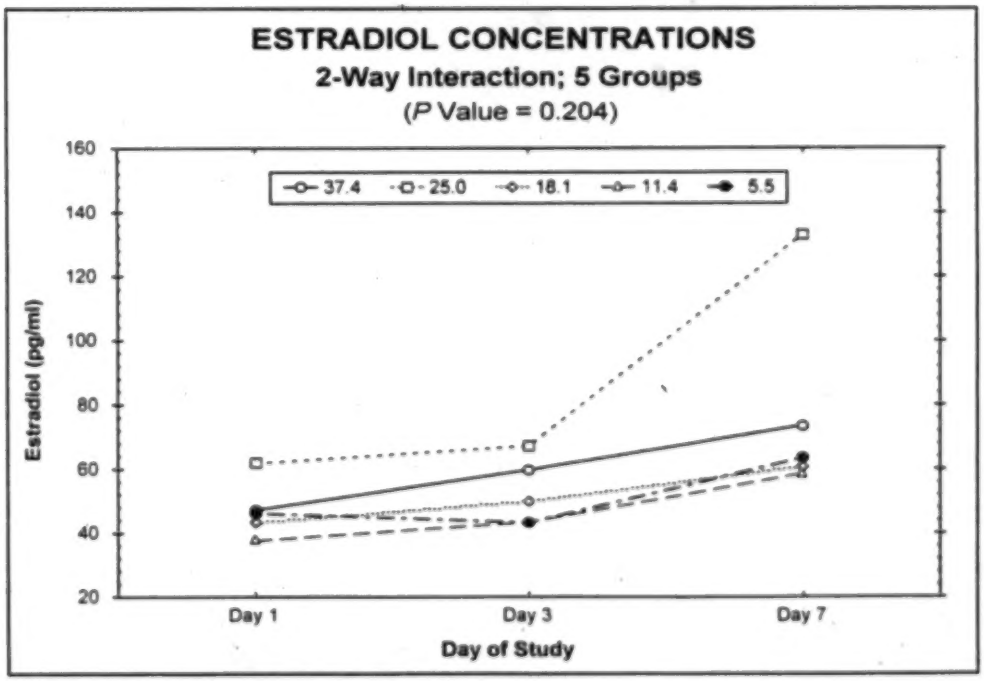

Figure 4.12 *Estradiol Concentrations (pg·ml^{-1})*
Inset defines energy availability group assignment (kcal·kg FFM^{-1}·day^{-1})

3. Thyroid Hormones

Throughout this results section, the hormonal data will be expressed as follows: i) the range of baseline values (average of days 1, 2 and 3); ii) the group mean at baseline; iii) the range of post-treatment values (Day 7); iv) the group mean at post-treatment; v) the statistical findings of baseline and post-treatment values; vi) the range in the absolute change from baseline to post-treatment values; vii) the group mean of the absolute change; viii) the range in the % change from baseline to post-treatment values; ix) the group mean of the % change; and x) the statistical findings of the absolute change and % change values.

a) Total - T_4 (Table 4.9; Figures 4.13 - 4.14)

Baseline values ranged from 3.55 to 7.56 $\mu g \cdot dL^{-1}$, with a group mean of 5.56 $\pm$ 0.2 $\mu g \cdot dL^{-1}$, and in post-treatment values from 3.84 to 8.30 $\mu g \cdot dL^{-1}$, with a group mean of 5.64 $\pm$ 0.2 $\mu g \cdot dL^{-1}$. Although there was an overall increase in T_4, there was no statistically significant main effect of time or interaction effect. Subjects ranged in the absolute change from baseline to post-treatment values from -1.35 to 1.30 $\mu g \cdot dL^{-1}$, with a group mean of 0.08 $\pm$ 0.1 $\mu g \cdot dL^{-1}$, and in the % change from baseline to post-treatment values from -21.8 to 22.0 %, with a group mean of 2.1 $\pm$ 1.9 %. There were no statistically significant differences between groups in the absolute change or % change in T_4.

Table 4.9 *Total - T_4 Concentrations ($\mu g \cdot dL^{-1}$)*

Energy Availability Group (kcal·kg FFM^{-1}·day^{-1})	Baseline	Post-Treatment	Δ	% Δ
Group 1: 37.4	5.81 ± 0.6	6.24 ± 0.8	0.43 ± 0.5	7.69 ± 7.8
Group 2: 25.0	5.28 ± 0.5	5.11 ± 0.5	-0.17 ± 0.2	-2.56 ± 2.9
Group 3: 18.1	6.04 ± 0.5	5.94 ± 0.5	-0.10 ± 0.2	-1.20 ± 3.0
Group 4: 11.4	5.59 ± 0.5	5.71 ± 0.4	0.12 ± 0.2	3.00 ±3.5
Group 5: 5.5	5.10 ± 0.3	5.28 ± 0.2	0.18 ± 0.2	4.48 ± 3.7
All Groups	5.56 ± 0.2	5.64 ± 0.2	0.08 ± 0.1	2.10 ± 1.9
All Groups (Range)	3.55-7.56	3.84-8.30	-1.35-1.30	-21.77-21.96
P Value			0.482	0.460

Values are means ± SE. Differences between groups were considered statistically significant when $p < 0.05$
No statistically significant main effect of time from baseline to post- treatment ($p = 0.412$)
No statistically significant main interaction effect between groups from baseline to post- treatment ($p = 0.482$)

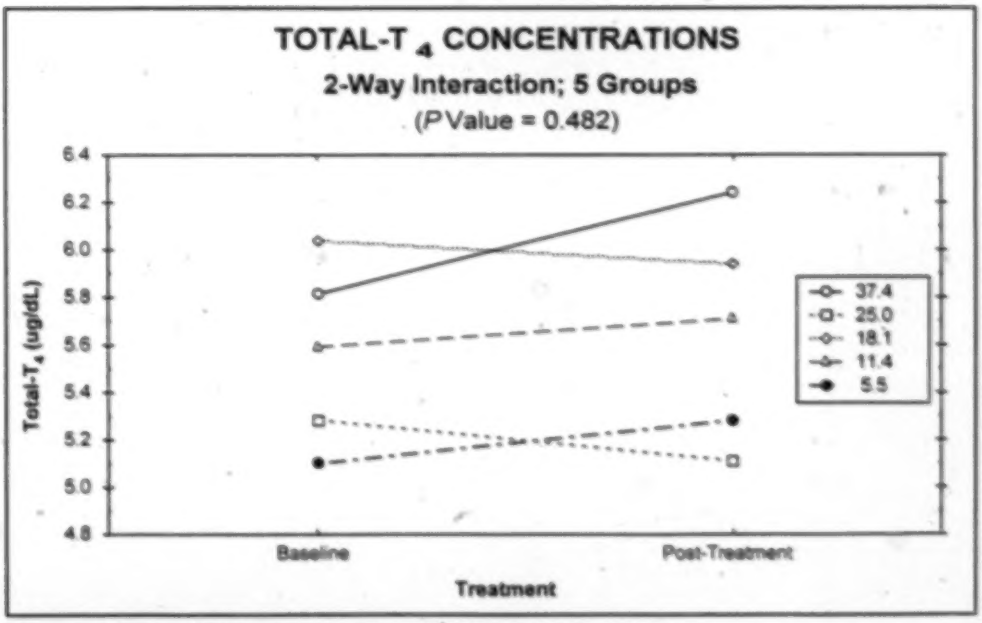

Figure 4.13 *Total-T_4 Concentrations ($\mu g \cdot dL^{-1}$): Baseline & Post-Treatment*
Inset defines energy availability group assignment (kcal·kg FFM^{-1}·day^{-1})

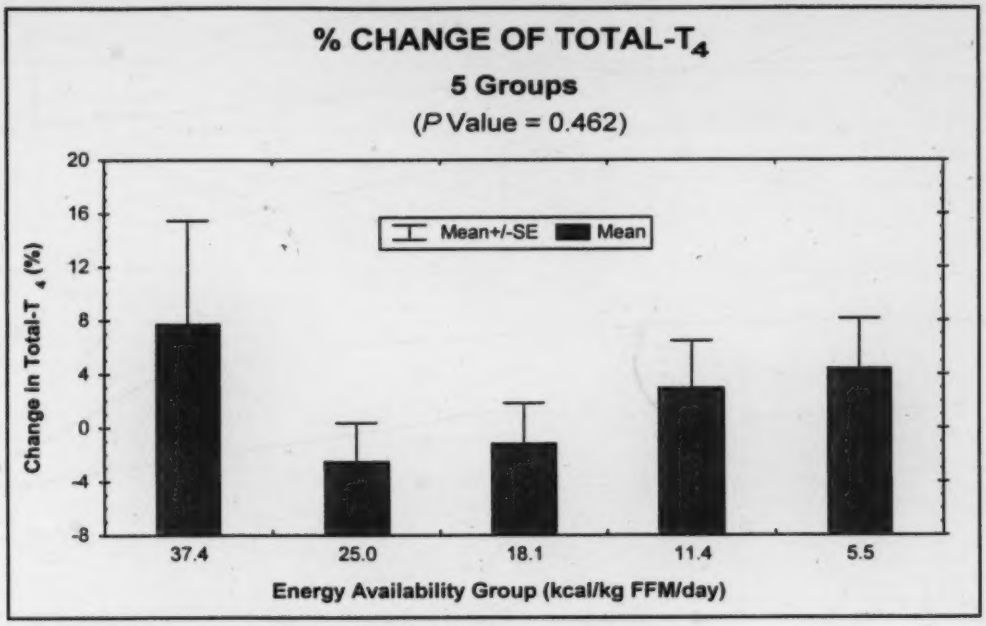

Figure 4.14 *% Change of Total-T_4 (from baseline to post-treatment)*

b) Total - T_3 (Table 4.10; Figure 4.15 - 4.16)

Baseline values ranged from 61.90 to 119.55 ng·dL^{-1}, with a group mean of 96.39 ± 2.4 ng·dL^{-1}, and in post-treatment values from 51.31 to 118.47 ng·dL^{-1}, with a group mean of 88.93 ± 2.7 ng·dL^{-1}. There was an overall significant decrease in T_3 (main effect of time), however, there was no interaction effect. Absolute change from baseline to post-treatment values ranged from -22.51 to 24.62 ng·dL^{-1}, with a group mean of -7.46 ± 2.0 ng·dL^{-1}, and in the % change from baseline to post-treatment values from -20.6 to 28.8 %, with a group mean of -7.5 ± 2.1 %. There were no statistically significant differences between groups in the absolute change or % change in T_3.

Table 4.10 *Total - T_3 Concentrations ($ng \cdot dL^{-1}$)*

Energy Availability Group ($kcal \cdot kg\ FFM^{-1} \cdot day^{-1}$)	Baseline	Post-Treatment	Δ	% Δ
Group 1: 37.4	95.87 ± 9.0	92.00 ± 11.3	-3.87 ± 5.7	-4.87 ± 6.0
Group 2: 25.0	97.52 ± 5.2	92.00 ± 2.9	-8.55 ± 3.1	-8.17 ± 2.8
Group 3: 18.1	99.86 ± 4.9	88.96 ± 4.1	-8.19 ± 4.3	-7.55 ± 4.5
Group 4: 11.4	92.30 ± 5.6	91.67 ± 7.8	-6.04 ± 6.4	-6.36 ± 7.3
Group 5: 5.5	96.32 ± 4.2	86.26 ± 3.6	-10.07 ± 3.9	-10.02 ± 3.7
All Groups	96.39 ± 2.4	88.93 ± 2.7 [a]	-7.46 ± 2.0	-7.48 ± 2.1
All Groups (Range)	61.90-119.55	51.31-118.47	-22.51-24.62	-20.57-28.84
P Value			0.911	0.966

Values are means ± SE. Differences between groups were considered statistically significant when $p < 0.05$
a = statistically significant main effect of time from baseline to post- treatment ($p = 0.002$)
No statistically significant main interaction effect between groups from baseline to post- treatment ($p = 0.911$)

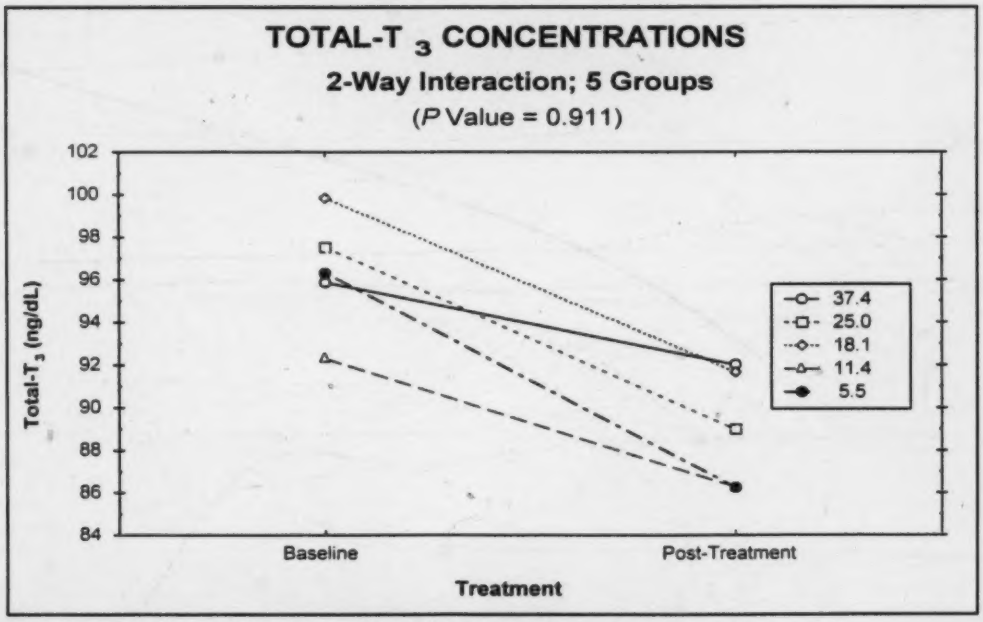

Figure 4.15 *Total-T_3 Concentrations ($ng \cdot dL^{-1}$): Baseline & Post-Treatment*
Inset defines energy availability group assignment ($kcal \cdot kg\ FFM^{-1} \cdot day^{-1}$)

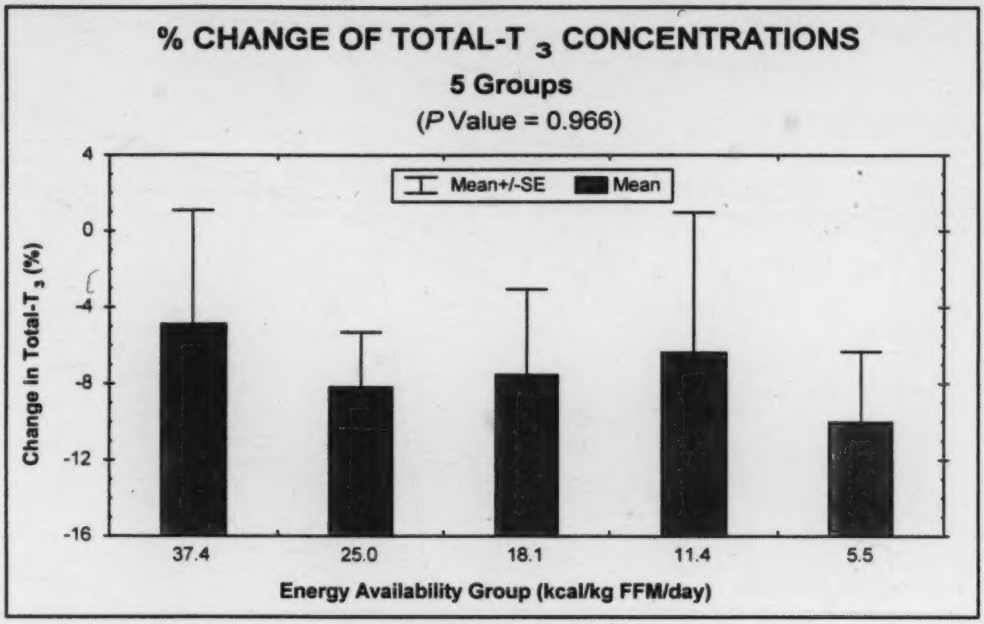

Figure 4.16 *% Change of Total-T_3 (from baseline to post-treatment)*

c) Free - T_4 (Table 4.11; Figures 4.17 - 4.18)

Baseline values ranged from 0.75 to 1.85 ng·dL^{-1}, with a group mean of 1.31 ± 0.1 ng·dL^{-1}, and in post-treatment values from 0.85 to 2.00 ng·dL^{-1}, with a group mean of 1.30 ± 0.1 ng·dL^{-1}. There were no statistically significant main or interaction effects. Absolute change from baseline to post-treatment values ranged from -0.64 to 0.32 ng·dL^{-1}, with a group mean of 0.00 ± 0.0 ng·dL^{-1}, and in the % change from baseline to post-treatment values from -35.9 to 38.9 %, with a group mean of 1.7 ± 2.6 %. There were no statistically significant differences between groups in the absolute change or % change in fT_4.

Table 4.11 *Free - T_4 Concentrations ($ng \cdot dL^{-1}$)*

Energy Availability Group ($kcal \cdot kg\ FFM^{-1} \cdot day^{-1}$)	Baseline	Post-Treatment	Δ	% Δ
Group 1: 37.4	1.54 ± 0.1	1.46 ± 0.2	-0.08 ± 0.1	-3.92 ± 8.2
Group 2: 25.0	1.37 ± 0.1	1.36 ± 0.1	0.00 ± 0.0	0.55 ± 3.7
Group 3: 18.1	1.45 ± 0.1	1.42 ± 0.1	-0.03 ± 0.1	-0.07 ± 6.3
Group 4: 11.4	1.22 ± 0.1	1.28 ± 0.1	0.06 ± 0.1	8.65 ± 6.9
Group 5: 5.5	1.00 ± 0.0	1.01 ± 0.0	0.02 ± 0.0	2.34 ± 4.9
All Groups	1.31 ± 0.1	1.30 ± 0.1	0.00 ± 0.0	1.73 ± 2.6
All Groups (Range)	0.75-1.85	0.85-2.00	-0.64-0.32	-35.89-38.87
P Value			0.801	0.694

Values are means ± SE. Differences between groups were considered statistically significant when $p<0.05$
No statistically significant main effect of time from baseline to post- treatment ($p = 0.898$)
No statistically significant main interaction effect between groups from baseline to post- treatment ($p = 0.801$)

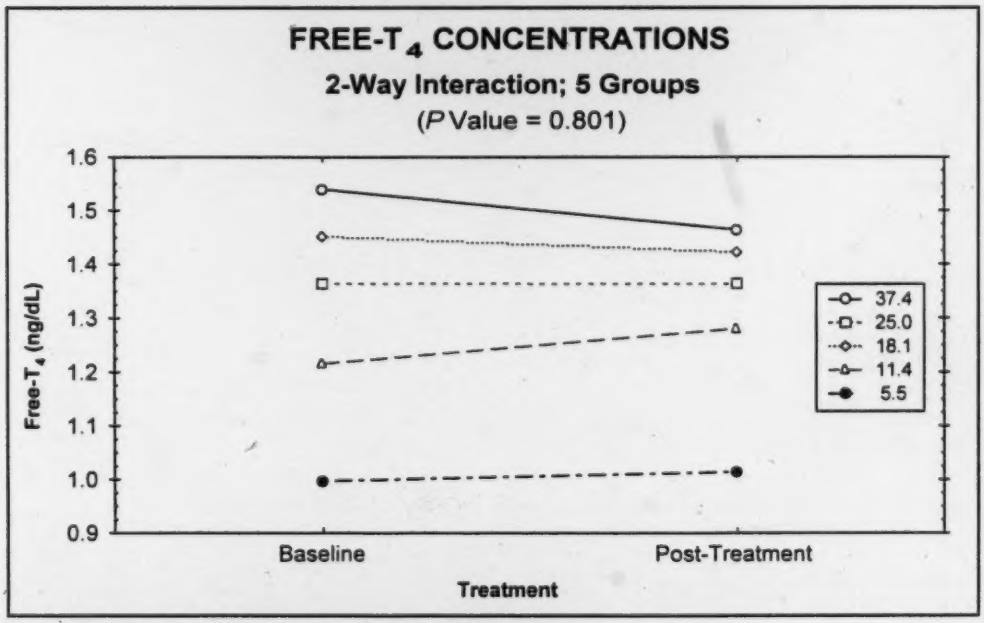

Figure 4.17 *Free-T_4 Concentrations: Baseline & Post-Treatment*
Inset defines energy availability group assignment ($kcal \cdot kg\ FFM^{-1} \cdot day^{-1}$)

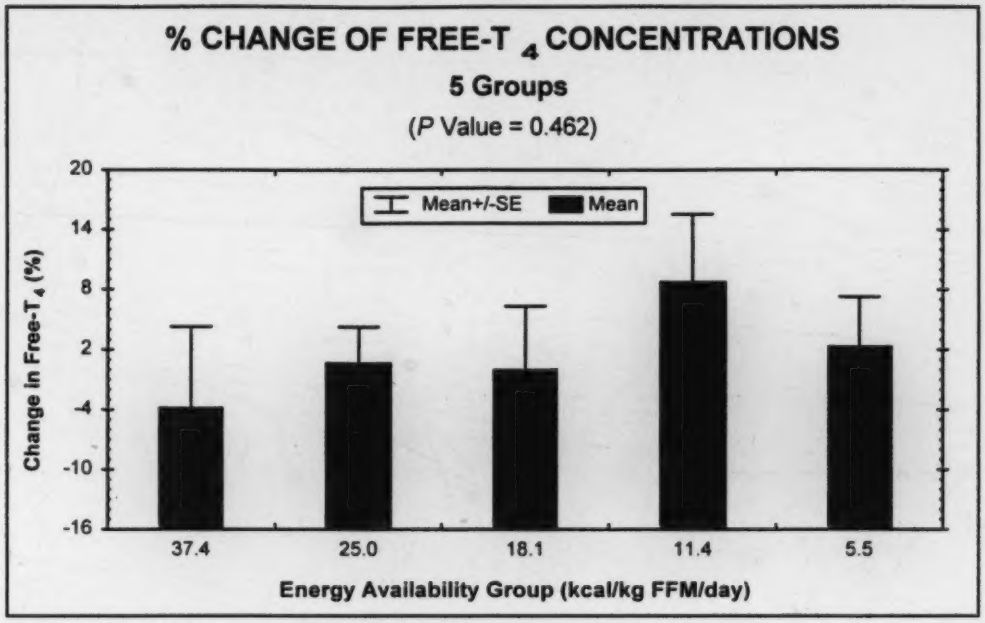

Figure 4.18 *% Change of Free-T_4 (from baseline to post-treatment)*

d) Free - T_3 (Table 4.12; Figures 4.19 - 4.20)

Baseline values ranged from 1.74 to 3.73 pg·ml^{-1}, with a group mean of 2.68 ± 0.1 pg·ml^{-1}, and in post-treatment values from 1.48 to 3.51 pg·ml^{-1}, with a group mean of 2.44 ± 0.1 pg·ml^{-1}. There was a statistically significant main effect of time from baseline to post-treatment with there being an overall decrease of fT_3. There was no interaction effect between groups. Absolute change from baseline to post-treatment values ranged from -0.83 to 0.49 pg·ml^{-1}, with a group mean of -0.25 ± 0.1 pg·ml^{-1}, and in the % change from baseline to post-treatment values from -29.8 to 17.9 %, with a group mean of -8.5 ± 2.5 %. There were no statistically significant differences between groups in the absolute change or % change in fT_3.

Table 4.12 *Free - T_3 Concentrations ($pg \cdot ml^{-1}$)*

Energy Availability Group ($kcal \cdot kg\ FFM^{-1} \cdot day^{-1}$)	Baseline	Post-Treatment	Δ	% Δ
Group 1: 37.4	2.83 ± 0.3	2.81 ± 0.3	-0.02 ± 0.2	-0.36 ± 5.7
Group 2: 25.0	2.80 ± 0.3	2.62 ± 0.1	-0.18 ± 0.2	-4.34 ± 5.6
Group 3: 18.1	2.79 ± 0.1	2.47 ± 0.1	-0.31 ± 0.2	-10.95 ± 5.4
Group 4: 11.4	2.53 ± 0.2	2.22 ± 0.1	-0.31 ± 0.2	-10.90 ± 5.5
Group 5: 5.5	2.49 ± 0.2	2.12 ± 0.2	-0.37 ± 0.1	-15.18 ± 5.6
All Groups	2.68 ± 0.1	2.44 ± 0.1[a]	-0.25 ± 0.1	-8.50 ± 2.5
All Groups (Range)	1.74-3.73	1.48-3.51	-0.83-0.49	-29.75-17.86
P Value			0.564	0.353

Values are means ± SE. Differences between groups were considered statistically significant when $p < 0.05$
a = statistically significant main effect of time from baseline to post- treatment ($p = 0.002$)
No statistically significant main interaction effect between groups from baseline to post- treatment ($p = 0.564$)

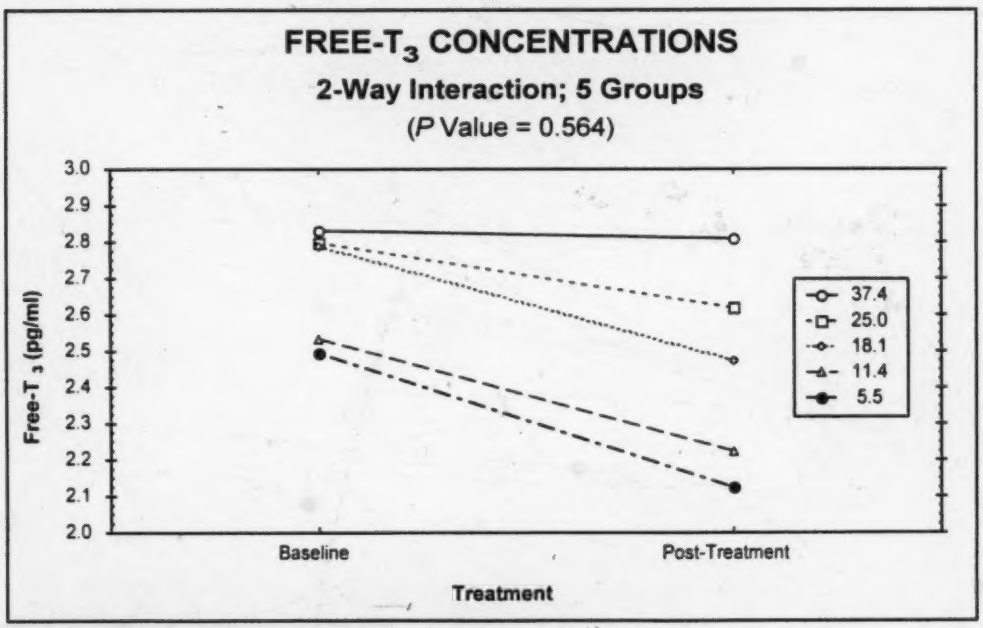

Figure 4.19 *Free-T_3 Concentrations ($pg \cdot ml^{-1}$): Baseline & Post-Treatment*
Inset defines energy availability group assignment ($kcal \cdot kg\ FFM^{-1} \cdot day^{-1}$)

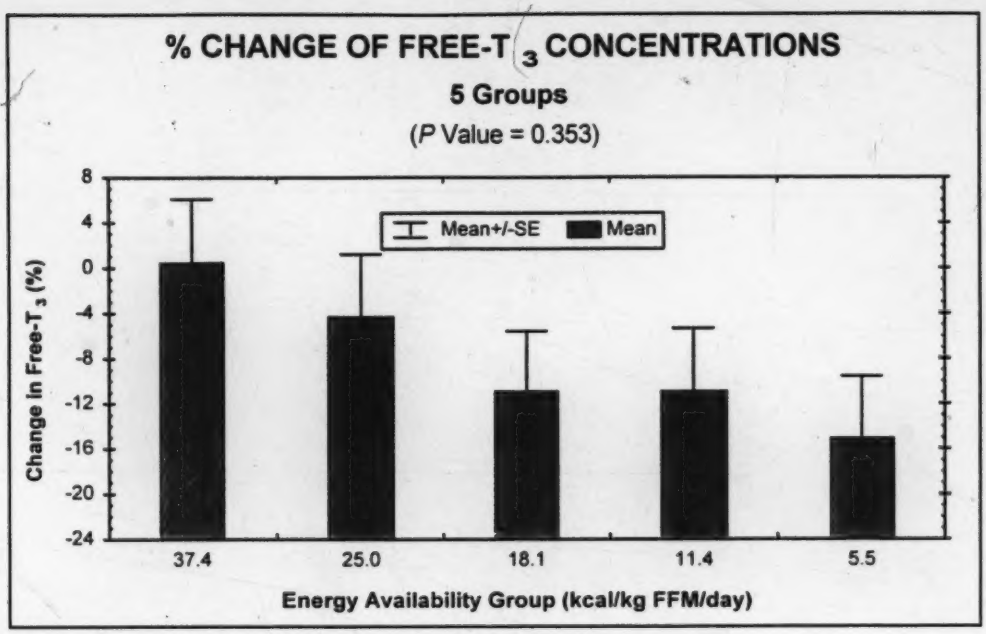

Figure 4.20 *% Change of Free-T_3 (from baseline to post-treatment)*

e) Reverse - T_3 (Table 4.13-4.14; Figures 4.21 - 4.22)

Baseline values ranged from 0.070 to 0.239 ng·ml^{-1}, with a group mean of 0.143 ± 0.01 ng·ml^{-1}, and in post-treatment values from 0.090 to 0.257 ng·ml^{-1}, with a group mean of 0.159 ± 0.01 ng·ml^{-1}. There was a statistically significant main effect of time from baseline to post-treatment with there being an overall increase of rT_3. Furthermore, there was also a statistically significant interaction effect between groups from baseline to post-treatment. Post-hoc analysis indicated that groups were different before treatment began. The following groups were different at baseline: 1 vs 4; 3 vs ALL; 5 vs ALL, and at post-treatment groups 3 and 5 were different from all groups. Absolute change from baseline to post-treatment values ranged from -0.050 to 0.057 ng·ml^{-1}, with a group mean of 0.016 ± 0.00 ng·ml^{-1}, and in the % change from baseline to post-treatment values from -29.9 to 67.9 %, with a group mean of 15.5 ± 4.0 %. There were statistically significant differences between groups in the absolute change from baseline to post-

treatment, these were: 1 vs 4; 1 vs 5; 3 vs 4; 3 vs 5. In addition, the % change in rT_3 for groups 1, 2, and 3 were different from groups 4 and 5.

Table 4.13 ***Reverse - T_3 Concentrations ($ng \cdot ml^{-1}$)***

Energy Availability Group ($kcal \cdot kg\ FFM^{-1} \cdot day^{-1}$)	Baseline	Post-Treatment	Δ	% Δ
Group 1: 37.4	0.159 ± 0.02	0.160 ± 0.02	0.001 ± 0.02	3.30 ± 11.43
Group 2: 25.0	0.147 ± 0.02	0.156 ± 0.02	0.009 ± 0.06	7.05 ± 5.11
Group 3: 18.1	0.178 ± 0.02	0.181 ± 0.02	0.002 ± 0.01	2.23 ± 3.08
Group 4: 11.4	0.132 ± 0.02	0.163 ± 0.02	0.031 ± 0.00	30.85 ± 9.75
Group 5: 5.5	0.104 ± 0.01	0.137 ± 0.01	0.033 ± 0.01	32.15 ± 6.63
All Groups[b]	0.143 ± 0.01	0.159 ± 0.01[a]	0.016 ± 0.00	15.52 ± 4.03
All Groups (Range)	0.070-0.239	0.090-0.257	-0.050-0.057	-29.89-67.95
P Value			0.032[c]	0.012[d]

Values are means ± SE. Differences between groups were considered statistically significant when $p < 0.05$
a = statistically significant main effect of time from baseline to post- treatment ($p = 0.001$)
b = statistically significant interaction effect between groups from baseline to post- treatment ($p = 0.032$)
* see Table 4.14 for differences between groups
c, d = see Table 4.14 for differences between groups

Table 4.14 ***Reverse - T_3 Statistically Significant Group Differences***

Statistically Significant Effect	Figure	Significant Differences between Energy Availability Groups (Post-Hoc: LSD)	
b	4.21	Baseline 1 vs 4; 3 vs ALL; 5 vs ALL	Post-Treatment 3 vs ALL; 5 vs ALL
c	-	1 vs 4; 1 vs 5; 3 vs 4; 3 vs 5	
d	4.22	1, 2, 3 vs 4, 5	

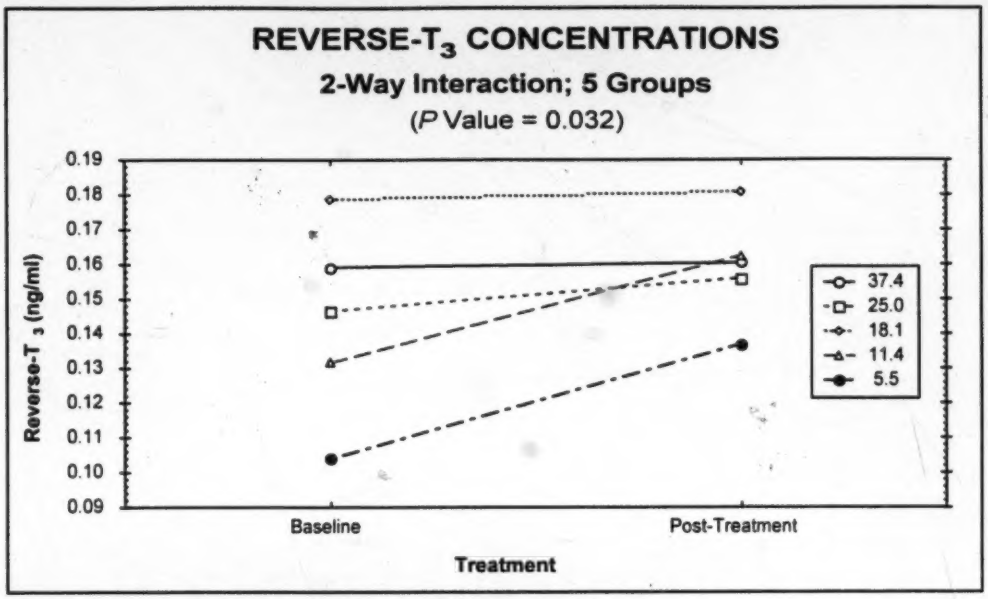

Figure 4.21 *Reverse-T_3 Concentrations (ng·ml^{-1}): Baseline & Post-Treatment*
Inset defines energy availability group assignment (kcal·kg FFM^{-1}·day^{-1})

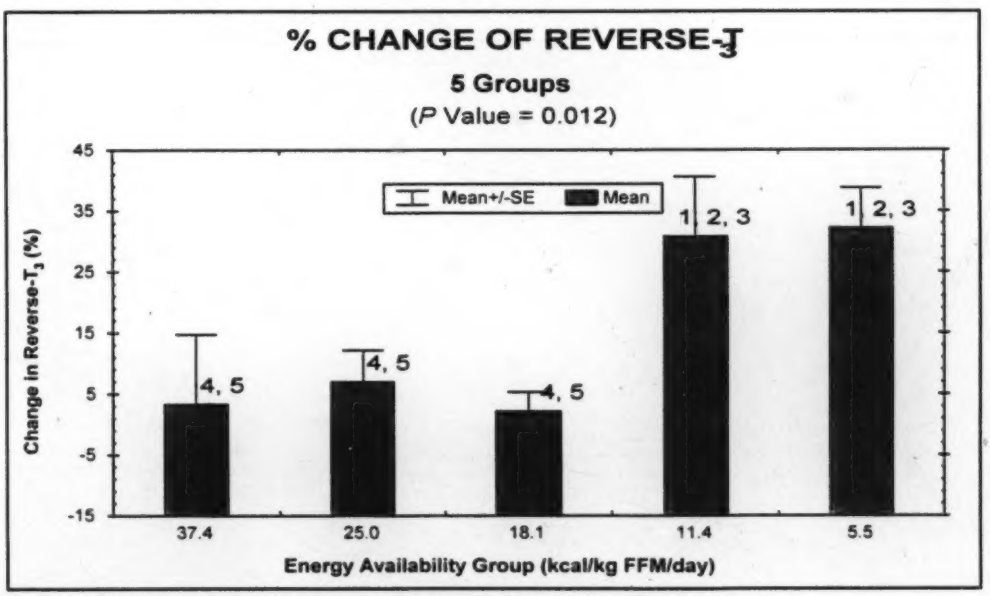

Figure 4.22 *% Change of Reverse-T_3 (from baseline to post-treatment)*
Matching numbers above means indicate which groups were different from each other ($P<0.05$)

f) Summary of Thyroid Hormone Findings

No statistically significant differences were found for T_4 and fT_4. Whereas, T_3 and fT_3 displayed an overall significant decrease with time (ie. main effect of time). Reverse-T_3 exhibited an overall significant increase with time and a statistically significant interaction effect between groups from baseline to post-treatment. Post-hoc analysis of the rT_3 data indicated that groups (ie. 1 vs 4; 3 vs ALL; 5 vs ALL) were different before treatment began and at post-treatment groups 3 and 5 were different from all groups. There were statistically significant differences between groups in the absolute change of rT_3 from baseline to post-treatment, these were: 1 vs 4; 1 vs 5; 3 vs 4; 3 vs 5. In addition, the % change in rT_3 for groups 1, 2, and 3 were different from groups 4 and 5.

g) % Change in T_3, rT_3 and Macronutrients

There were no correlations found between the % changes in T_3 and rT_3 and the % changes in macronutrients (ie. CHO, fat and protein) in the subjects diets. The % changes in macronutrients were the calculated differences between the subjects usual diet and assigned 4-day treatment diet (ie. 55% CHO, 30% fat and 15% protein).

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CHAPTER V

DISCUSSION

Effects of Energy Availability on Thyroid Metabolism

Previous studies have shown that energy availability, rather than dietary energy intake or exercise energy expenditure alone, are the factors affecting thyroid metabolism in habitually sedentary women (Loucks & Callister, 1993; Loucks & Heath, 1994a). That is, 'lowered T_3 state' (ie. reductions in T_3 and fT_3 with an increase in rT_3 levels) have been induced by the energy cost of exercise and prevented by increasing dietary energy intake. In this study which examined the effects of 4-days of varying energy availabilities in physically trained women, 'lowered T_3 state' was induced. Loucks & Heath (1994a) identified thresholds in which reductions in T_3 and fT_3 occurred abruptly between 19.0 and 25.0 kcal·kg LBM^{-1}·day^{-1} and increases in fT_4 and rT_3 occurred between 10.8 and 19.0 kcal·kg LBM^{-1}·day^{-1}.

(a) Significant Findings

Main effect of time results indicated that the present study group experienced 'lowered T_3 state'. That is, T_3 and fT_3 levels for the entire group ($n=29$) decreased significantly with time, while rT_3 levels increased significantly with time. The induction of 'lowered T_3 state' has been elicited in sedentary women by Loucks & Heath (1994a). However, in contrast to the present study, 'lowered T_3 state' was induced only in the lowest energy availability group (ie. 10.8 kcal·kg LBM^{-1}·day^{-1}) (Loucks & Heath, 1994a). Since T_3 and fT_3 levels dropped and rT_3 levels increased in the entire research group, these findings might suggest that

the physically trained woman may be more susceptible to the induction of 'lowered T_3 state' across the varying energy availabilities than the sedentary woman.

In contrast to Loucks & Heath (1994a) results, statistical analysis in the present study indicated that there was only a single thyroid threshold for the physically trained woman, which occurred at a similar energy availability level as Loucks & Heath. In the present study, rT_3 abruptly increased between 11.4 and 18.1 kcal·kg FFM^{-1}·day^{-1}. Loucks & Heath (1994a) identified a threshold for rT_3 in which hormonal levels were abruptly increased by 26%, when going below the threshold (ie. threshold between 10.8 and 19.0 kcal·kg LBM^{-1}·day^{-1}). Whereas, in the present study, rT_3 increased by 31% when going below the identified threshold.

The rT_3 threshold (with respect to the level of energy availability) which was identified in the present study, is similar to the threshold identified by Loucks & Heath (1994a). In addition, examining the data from Spaulding et al. (1976) and O'Brian et al. (1980), in which only dietary intake was manipulated in obese subjects, it was found that rT_3 rose at a threshold of dietary energy intake, with dietary CHO content playing no role in the position of this threshold. The results of Spaulding et al. (1976) and O'Brian et al. (1980) indicated that there was no effect on rT_3 levels when energy intake was above 800 kcal·day^{-1} (Spaulding et al., 1976), but that, rT_3 levels increased when energy intake was $\leq$ 600 kcal·day^{-1}(O'Brian et al., 1980). Unfortunately, body composition data (ie. FFM) were not given for either of these studies, and therefore specific energy availabilities can not be determined. The energy availabilities for subjects in this present study were as follows: 260, 576, 889, 1238 and 1715 kcal·day^{-1} (in descending group order), with

the rT_3 threshold identified between 576 and 889 kcal·day^{-1}. Therefore, the results from this study suggest that the effects of energy availability on rT_3 metabolism observed in physically trained non-obese women, compared to habitually sedentary non-obese women (Loucks & Heath, 1994a) and habitually sedentary obese individuals (O'Brian et al., 1980; Spaulding et al., 1976) are all very similar. Moreover, regardless of a woman's training state or fitness level, lower energy availabilities appear to influence rT_3 levels in a similar fashion.

Due to T_4 levels remaining relatively constant for all energy availability groups in the present study and in Loucks & Heath's (1994a) study, it appears as though 'lowered T_3 state' is induced by changes in the peripheral activities of thyroid metabolism as T_3 levels were reduced despite the adequacy of T_4 as a precursor. Moreover, Loucks & Callister (1993) found a statistically significant increase in T_4 levels in their energy-deficient groups. This outcome suggests that hypothalamic sensitivity to the negative feedback of a reduction in T_3 remained intact, whereby increasing thyroid production of T_4 in the habitually sedentary women. Since *Type I 5'-deiodinase and Type II 5'-deiodinase* catalyse the deiodination of both T_4 to T_3, and rT_3 to 3,3'-T_2, it appears as though the activity of one or both of these enzymes may have decreased leading to the development of 'lowered T_3 state' (ie. less T_3 was produced and there was a reduction in rT_3 clearance). It is described that most circulating T_3 is derived via hepatic deiodination of T_4 by the Type I 5'-deiodinase (Kaplan, 1984), which has its highest levels of activity in the thyroid, kidney and liver (Leonard & Koehrle, 1996). Whereas, it appears that Type II 5'-deiodinase generates T_3 for local use in organs such as the brain, pituitary and brown adipose tissue (Kaplan, 1984; Leonard & Koehrle, 1996).

Loucks & Heath (1994a) accounted for rT_3 having a lower threshold due to the elevated fT_4 levels presented to peripheral 5'-deiodinase and a reduced clearance rate of rT_3 to 3,3'-T2 by 5'-deiodinase. However, peripheral 5'-deiodinase conversion does not occur between fT_4 and rT_3. This conversion is completed by 5-deiodinase. Furthermore, no explanation was given for fT_4 having a lower threshold. An alternative explanation may be that one (or more) of the deiodinase enzymes has its own energy availability threshold, in which certain activities account for fT_4 and rT_3 to increase.

Another enzyme, *Type III 5-deiodinase* may also play a role in inducing 'lowered T_3 state'. Type III appears in most tissues except the anterior pituitary, and has its highest levels of activity in the liver, brain and placenta. It is one of the major routes for T_3 degradation to 3,3'-T_2, T_4 to rT_3 and rT_3 to 3',5'-T_2 (Kaplan, 1984; Leonard & Koehrle, 1996). With an increase in enzymatic activity for the conversion of T_3 to 3,3'-T_2 and T_4 to rT_3, this could account for 'lowered T_3 state'. However, LoPresti et al. (1991) found that the rise of serum rT_3 levels appears to be a consequence of a decreased rT_3 clearance, as peripheral conversion of T_4 to rT_3 remains unchanged. Furthermore, if there was an increase in Type III activity for the conversion of rT_3 to 3',5'-T_2, rT_3 levels would decrease. Loucks & Callister (1993) predict that the impairment of 5'-deiodinase activity and the enhancement of 5-deiodinase activity together, with the inhibition of active transmembranous transport of T_3 and T_4 will prove to be regulated by energy availability. Despite the importance of this inactivation pathway (ie. Type III deiodinase), only relatively recently has any information been obtained about it (Leonard & Koehrle, 1996). Speculations are advanced that modulation of Type II deiodination in the pituitary and hypothalamus may serve as a defence against

thyroid hormone deficiency, that Type III deiodination may act together with Type II deiodination to control brain T_3 content, and that local rT_3 production in the brain by the Type III pathway might have some, as yet unidentified, physiological significance (Kaplan, 1984).

(b) Trends

The previous section discussed statistically significant findings for T_3, fT_3 and rT_3 levels with respect to the 'main effect of time' and rT_3 displaying significant differences between energy availability groups. Although not significant, fT_3 and fT_4 responded in a similar fashion to other studies examining diet and energy availability and thyroid metabolism. For both fT_3 and fT_4, changes in physically trained women were similar to those observed in untrained women (Loucks & Heath, 1994a). Loucks & Heath (1994a) identified a threshold for fT_3 in which hormonal levels were abruptly reduced by 9%, whereas, fT_4 levels were abruptly increased by 12%, when going below their respective thresholds. In comparison, fT_3 decreased by 11% (between 18.1 and 25.0 kcal·kg FFM^{-1}·day^{-1}) and fT_4 increased by 9% (between 11.4 and 18.1 kcal·kg FFM^{-1}·day^{-1}) in the current study. These hormonal responses in physically active women were comparable to Loucks & Heath's (1994a) habitually sedentary women. Further research is necessary to clarify the possible existence of thresholds at varying energy availabilities for the physically trained woman. Moreover, the absence of identifying additional thyroid thresholds may be due to a small sample size or the effects of training, in which the trained subject appears to become more susceptible to the induction of 'lowered T_3 state' across the varying energy availabilities.

Loucks and Heath (1994a) identified energy availability thresholds for T_3, fT_3, fT_4 and rT_3. Another possible explanation for the absence of statistically significant thresholds for T_3, fT_3 and fT_4 in the present study investigating physically trained women is that the subjects average 7-day habitual caloric intakes were similar to Loucks & Heath's (1994a) sedentary women (ie. 2,295 vs 2,259 kcal·day^{-1}, respectively). Loucks & Heath's (1994a) subjects all had $\dot{V}O_{2max} < 43$ ml·kg^{-1}·min^{-1} and had performed <60 min of habitual aerobic activity a week for the previous 3 months. Conversely, the participants in this study had at least a $\dot{V}O_{2max}$ of 44.8 ml·kg^{-1}·min^{-1} and performed approximately 4 hours of aerobic activity a week. This suggests that the habitual energy intake of the women in the present study were similar to those in the study of Loucks & Heath's (1994a), however, the trained women were expending many more calories through exercise. Therefore, the physically active women in this study were at a lower habitual energy availability versus Loucks & Heath's (1994a) sedentary women, who were at a habitual energy availability of approximately 50.6 kcal·kg LBM^{-1}·day^{-1} (2259 kcal·day^{-1}). In comparison, assuming that each participant in the present study exercised the aerobic equivalent of running 8 km·day^{-1} and habitually consumed 2295 kcal·day^{-1}, this would equate to an energy availability of 36.9 kcal·kg FFM^{-1}·day^{-1} (1795 kcal·day^{-1}). It is noteworthy that this level of energy availability is similar to the energy availability that Group I experienced throughout the 4-days of treatment. In retrospect, a higher energy availability group could have been created in the present study, to examine the effects of a higher energy availability than the subjects were used to. However, most subjects in Group I experienced a difficult time trying to consume all cans of drink given to them each day, and therefore increasing the amount of calories may have been

difficult. Statistical analyzes indicate that Group I experienced minimal changes in T_3, fT_3 and rT_3 levels, which suggests that this may have been the energy availability that these subjects were adapted to.

All groups experienced reductions in T_3 (4.9 - 10.0 %) and fT_3 (0.4 - 11.0 %) levels, however, no group experienced a drastic enough change for a threshold to be identified. It may be possible that T_3 and fT_3 share the same threshold and the difficulty in finding a threshold in the physically trained EUM woman, is due to the fact that T_3 and fT_3 levels have been chronically reduced, by habitual energy deficiency. Therefore, it becomes it more difficult to elicit or identify a threshold, due to the reduced levels. Trends have been found in the EUM physically trained woman to suggest that fT_3 levels are reduced compared to EUM sedentary controls (Baer, 1993; Loucks et al., 1992; Marcus et al., 1985). In addition, reduced T_3 levels have been found in athletic EUM women compared to EUM sedentary controls, with significant differences found by Harber et al. (1998) and no significant differences (though lower levels) found by Loucks et al. (1992) and Marcus et al. (1985). If in fact there is a fT_3 threshold in physically trained women between energy availabilities 18.1 and 25.0 kcal·kg FFM^{-1}·day^{-1}, this threshold is similar to the threshold identified by Loucks & Heath (1994a) for both fT_3 and T_3 (ie. 19.0 and 25.0 kcal·kg LBM^{-1}·day^{-1}).

In studies examining the effects of dietary restriction in sedentary obese individuals, T_3 levels declined when dietary energy intake was below a particular threshold, with the CHO content of the diet influencing the location of this threshold. Spaulding et al. (1976) found that T_3 levels declined when energy intake was reduced to 800 kcal·day^{-1} if CHO content was $<$ 200 kcal·day^{-1}. However, when energy intake was reduced to $\leq$ 600 kcal·day^{-1}, T_3 levels fell regardless of CHO content (O'Brian et al., 1980). In contrast

to the results of O'Brian et al. (1980) and Spaulding et al. (1976) the T_3 threshold values of Loucks & Heath (1994a) were somewhat different. Levels of T_3 were reduced in Loucks & Heath's (1994a) study between energy availabilities of 850 and 1140 kcal·day^{-1}, with the CHO content of each diet consisting of 468 and 627 kcal·day^{-1}, respectively. Therefore, there appears to be some discrepancy between the identification of a T_3 threshold, and perhaps this depends on the population (ie. habitually sedentary non-obese vs habitually sedentary obese). However, a factor that may contribute to this discrepancy in trying to identify a T_3 threshold is that the non-obese habitually sedentary women studied by Loucks & Heath (1994a) derived their energy availability status through the combination of diet and exercise, whereas the obese habitually sedentary subjects' energy availability levels were determined through diet alone (see *'Effects of Energy Availability on Substrate Utilization'* for an explanation of how these differences may play a role in identifying a threshold).

Thyroid Baseline Findings

A comparison of thyroid hormone concentrations is presented in Table 5.1. Variability in hormone levels between the studies listed in Table 5.1 may exist due to different RIA kit specifications. All of the thyroid hormone concentrations in the present study fit in the manufacturers RIA kits 'normal range'. An interesting finding with the subjects in the current study, is that T_3 levels at baseline were between Harber et al.'s (1998) EUM and AMEN trained women and very similar to Loucks et al.'s (1992) AMEN group. In addition, fT_3 levels in the current study were between Loucks et al. (1992) EUM and AMEN athletes. Perhaps

the EUM group of women who participated in the present study were at a lower energy availability than the EUM trained subjects in Harber et al. (1998) and Loucks et al. (1992) studies.

Table 5.1 ***Comparison of Circulating Thyroid Hormone Levels***

Study	Population	T_4 ($\mu g \cdot dL^{-1}$)	T_3 ($ng \cdot dL^{-1}$)	fT_4 ($ng \cdot dL^{-1}$)	fT_3 ($pg \cdot ml^{-1}$)	rT_3 ($ng \cdot ml^{-1}$)
Foster	EUM Trained; Baseline	5.56 ± 1.2	96.4 ± 13.1	1.31 ± 0.3	2.68 ± 0.5	0.14 ± 0.1
(current study)	EUM Trained; Treatment	5.64 ± 1.2	88.9 ± 14.3[a]	1.30 ± 0.3	2.44 ± 0.5[a]	0.16 ± 0.0[a]
Harber et al.	Anorexic	4.8 ± 0.4[b]	84.8 ± 15.3[b]	n/a	n/a	n/a
(1997)	EUM, Untrained	7.0 ± 1.0	118.9 ± 12.4	n/a	n/a	n/a
Harber et al.	AMEN; Trained	5.40 ± 0.3[c]	91.2 ± 6.5[c]	n/a	n/a	n/a
(1998)	EUM; Trained	6.10 ± 0.3[d]	104.2 ± 6.5[d]	n/a	n/a	n/a
	EUM; Nonathlete	7.00 ± 0.3	117.2 ± 6.5	n/a	n/a	n/a
Loucks et al.	AMEN Athlete	6.0 ± 0.3[e]	97.7 ± 6.5[f]	0.94 ± 0.1[g]	2.52 ± 0.1[g]	0.16 ± 0.0[h]
(1992)	EUM Athlete	6.1 ± 0.3[e]	108.1 ± 5.2	1.23 ± 0.1	3.25 ± 0.1	0.19 ± 0.0
	EUM Sedentary	7.0 ± 0.3	127.0 ± 5.9	1.25 ± 0.1	3.39 ± 0.2	0.18 ± 0.0
Loucks et al. (1992)	Normal Ranges	5.67-11.27	71.6-201.8	0.8-2.7	2.6-4.8	0.10-0.50
From RIA Kits	Normal Ranges	4.5-12.5	86-187	0.8-2.0	1.4-4.4	0.09-0.35

Values are means ± SD.
a = statistically significant main effect of time from baseline to post-treatment ($p<0.05$)
b = statistically significant from EUM untrained ($p<0.05$)
c = statistically significant from EUM trained and EUM nonathlete ($p<0.05$)
d = statistically significant from nonathlete ($p<0.05$)
e = statistically significant from EUM sedentary ($p<0.05$)
f = statistically significant from EUM sedentary ($p<0.01$)
g = statistically significant from EUM athlete and EUM sedentary ($p<0.01$)
h = statistically significant from EUM athlete ($p<0.05$)

Effects of Energy Availability on Substrate Utilization

Loucks & Callister (1993) and Loucks & Heath (1994a) suggest that induction of 'lowered T_3 state' may be due to CHO availability and/or free fatty acid mobilization, and that their proportions may be potential regulators of peripheral Type I 5'-deiodinase activity. Due to the discrepancies found between sedentary and exercising subjects when attempting to identify a T_3 threshold (Loucks & Heath, 1994a; O'Brian et al., 1980; Spaulding et al., 1976), an examination of the effects of energy availability on CHO and fat utilization is warranted.

In the present study, regardless of the energy availability group, an interaction was found for the RER between the day of exercise (ie. Day 1, 2, 3 and 4) and the exercise bout (ie. first, third, last one). RER values were highest on the first day of exercise, during the first bout of exercise, whereas RER values were the lowest on the last day of exercise, during the last bout of exercise. As the study progressed, the subjects CHO stores were likely progressively depleted, which were accompanied by an increase in fat utilization. In addition, both exercise days 1 and 4 displayed similar changes in RER values, such that, as the exercise progressed throughout each day, RER values decreased. The remaining RER data are difficult to interpret. Changes that occurred on Day 2 and 3 were unexpected, as Day 2's RER values decreased from the first exercise bout to the third exercise bout, and then increased during the subject's last exercise bout. Conversely, Day 3's RER values displayed the opposite effect, in which RER values increased from the first exercise bout to the third exercise bout, and then decreased during the last bout of exercise. What is most interesting for Days 2 and 3's RER results, is that all groups followed these similar trends, besides Group 5 on day 3, in which the RER values were relatively stable. A confounding variable which could have played a role in affecting RER values is the time in which participants consumed their liquid nutritional supplement

during the exercise bout. However, this variable can not be examined as there were no regulations on the time at which the supplement was consumed.

In the present study, the mean RER values for the 4-days of treatment ranged from an RER for Group 1 of 0.93 (77.4 % CHO; 22.6% fat) to an RER for Group 5 of 0.88 (60.8% CHO; 39.2% fat). One can see that Group 5 subjects utilized approximately 16.6% less CHO (and 16.6% more fat) compared to Group 1. In fact, each energy availability group utilized different amounts of CHO and fat, with an increase in fat utilization as energy availability decreased. Loucks & Heath (1994a) did not provide specific RER values for each energy availability group, and therefore it is difficult to ascertain whether there were differences between their groups in terms of CHO and fat utilization. Moreover, on an individual basis, the RER range indicates a difference of 26.6% in substrate utilization. These differences (for both groups and individuals) may even be greater, due to the fact that during ketosis (which may have started to occur in subjects in Group 5) RER values will be artificially inflated. Loucks & Heath's (1994a) range for CHO utilization was 62 to 88%, indicating that their subjects utilized more CHO stores versus the women who participated in the present study, who had a range of 54 to 81% CHO utilization. This comparative range is somewhat misleading, due to the fact that the present study implemented an additional lower energy availability group. Therefore, when the RER values from the lowest energy availability group were eliminated (from the present study), the range for CHO utilization was 61 to 81%.

There are potentially 2 explanations for why the physically trained women have a slightly greater percentage of fat utilization and lower CHO utilization than the habitually sedentary women. Loucks & Heath's (1994a) subjects were exercising at a higher intensity (70% $\dot{V}O_{2max}$), whereas the women in this study exercised at approximately 90% VT (38.9% to 66.2% $\dot{V}O_{2max}$). This could explain the greater utilization of CHO stores in the habitually sedentary women, due to the fact that at a higher exercise

intensity, more CHOs are utilized (Foss & Keteyian, 1998). The second explanation is that these women were physically trained, and therefore the ability of the muscles to oxidize fat was improved as a result of endurance training. The increase in the muscle's capacity to oxidize fat following endurance training is partly related to the ability of endurance-trained muscle to store more fat (triglycerides) than untrained muscle (Foss and Keteyian, 1998). In addition, in the trained muscle, there is an increase in the activities of the enzymes involved in βoxidation of fat or other biochemical pathways. This leads to increased use of fat as an energy source, sparing glycogen (Wilmore & Costill, 1999).

Reproductive function examined in animal models indicate a dependence not entirely on energy availability, nor upon CHO intake and availability, but more precisely upon glucose availability to the brain (Wade & Schneider, 1992). In the physically active women, there appeared to be an overall slight increase in fat utilization and a decrease in CHO utilization which may potentially enhance glucose availability to the brain. Therefore, it appears that if a woman wants to decrease her energy availability in an attempt to lose weight (ie. decrease energy intake or increase exercise energy expenditure to create a similar deficit), exercise may be protective in nature in maintaining reproductive function. In that, fat stores will be utilized to a greater degree during exercise versus caloric restriction and therefore, enhance glucose availability to the brain (Loucks et al., 1998b). However, if free fatty acid mobilization is a trigger to the body that available energy is low, then exercise may not act in the same protective manner.

Other studies by Loucks et al. (1994b; 1994c; 1995) have also suggested that exercise may be protective in nature. Loucks et al. (1994b; 1994c; 1995) examined the effects of 2 extreme energy availabilities (ie. balanced vs deficient) in habitually sedentary women on LH pulsatility. The low energy availability group (ie. 10.0 kcal·kg LBM^{-1}·day^{-1}) was established through severe energy restriction alone, through severe exercise energy expenditure alone and through the combination of moderate caloric

restriction and moderate exercise energy expenditure. All low energy availability groups experienced suppressed LH pulsatility (ie. increase in LH amplitude and decrease in LH pulse frequency). However, the smallest effect was seen in women whose low energy availability was due to exercise alone. Loucks et al. (1998b) have noted that these results are inconsistent with an implicit assumption of the energy availability hypothesis which states that an increase in energy expenditure and a similar reduction in energy intake would have similar effects on LH pulsatility. Therefore, this suggests that contrary to popular belief, exercise may actually be protective against the disruption of LH pulsatility by low energy availability.

Even though the physically trained women in the present study utilized a slightly different ratio of substrates during exercise compared to Loucks & Heath's (1994a) study, it appears as though these differences did not have an impact on the level of CHO availability and/or free fatty acid mobilization that could result in a different fT_3 threshold identified by Loucks & Heath (1994a) compared to the one that has been suggested to exist in the present study. If perhaps Loucks & Heath (1994a) and the present study had implemented more levels of energy availability (ie. more groups) over the same range of energy availability examined (ie. a smaller difference between groups) it is possible that two different fT_3 thresholds between both studies may have been identified, due to the difference in substrate utilization. These results suggest that the fT_3 threshold (18.1 to 25.0 kcal·kg FFM^{-1}·day^{-1}) may be in the middle of the threshold identified, versus at the extreme ends within the energy availability threshold range.

Effects of Energy Availability on Weight Loss

There also appeared to be a relationship between energy availability and body weight loss. In this study, Groups 1, 2 and 3 lost approximately 1% (0.8 kg) of their body weight, whereas Groups 4 and 5 lost almost 4% (3.9 kg) and 3% (2.9 kg) of their body weight, respectively. This suggests that there may be a

body weight loss threshold between energy availabilities of 11.4 and 18.1 kcal·kg FFM^{-1}·day^{-1}. Unfortunately, no comparison can be made with Loucks & Heath's (1994a) study, as no weight changes were reported. However, weight loss has been reported in a similar study (Loucks et al., 1998b) in which energy availabilities were set at a low (ie. deficient; 10 kcal·kg LBM^{-1}·day^{-1}) and high (ie. balanced; 45 kcal·kg LBM^{-1}·day^{-1}) level. The low energy availability group was established in similar fashion as Group 4 in the present study (ie. similar in caloric intake and exercise energy expenditure based on kcal·kg LBM^{-1}·day^{-1}). Whereas, Loucks et al's (1998b) high energy availability group had a slightly greater caloric intake (ie. 5 kcal·kg LBM^{-1}·day^{-1}) and therefore a slightly higher energy availability compared to the present study. Loucks et al. (1998b) found that the low energy availability group of EUM habitually sedentary women lost 1.7 kg (2.8%) of their body weight, whereas there was no change in the high energy availability groups body weight.

The weight loss that subjects experienced in this study could be attributed to a loss of fat or muscle or water loss or any combination of these, over the 4 days of treatment. Subjects were routinely reminded to consume water even when they were not thirsty. A sign that most subjects were consuming sufficient water, was that many had to frequently visit the restroom to urinate between each or every other exercise bout. Each group may have experienced some degree of water and perhaps muscle loss, with Groups 4 and 5 experiencing this to a greater degree. Although the relative amount of exercise performed was the same for each group, Groups 4 and 5 would likely have experienced the greatest reduction in CHO stores, with an accompanying loss of water due to the reduced caloric intakes. With every gram of CHO used by the body, approximately 3 g of water are lost (Wilmore & Costill, 1999). The total body CHO content is about 375 g to 475 g, and therefore depletion of the stored CHO would result in a loss of approximately 1,125 g to 1,425 g of water, which equates to approximately 1.1 kg to 1.4 kg of body weight (Felig & Wahren, 1975).

Furthermore, although not identified in urinary testing for most subjects, some degree of ketosis may have started and this also exaggerates water loss (Rhoades & Tanner, 1995). Ketosis was observed in 1 subject in Group 5, who lost 0.8 kg or 1.5% of her body weight. This subject experienced 'moderate' amounts of acetoacetic acid in her urine, and complained about the noticeable unpleasant alteration of her breath on Day 7/1.

Another factor to take into consideration when examining weight loss, is whether it has occurred in a trained or untrained individual. The subjects in this study were trained, and research has found that with adequate rest and sufficient dietary CHOs, trained muscle stores considerably more glycogen than untrained muscle (Abernethy et al., 1990). Therefore, this may be a reason to see more dramatic changes in weight if CHO stores become depleted in a trained individual, as more CHO may be depleted from the muscles and therefore a greater loss of water will result. Due to this increase in glycogen stored in trained muscles, this may have played a role in only one subject becoming ketotic (in Group 5) in the study. It is possible that this subject started with a lower level of glycogen in her muscles at the beginning of the study. Loucks (1993, 1994a) indicates that in her studies, the urinary ketone acetoacetic acid levels were monitored as an indicator of compliance in her under-compensated groups, and therefore this suggests that these participants became ketotic. However, after personal communication with Loucks, she indicated that a β-hydroxybutyrate assay was performed to examine ketoacidosis which was used to determine compliance of her subjects.

Energy Availability, Thyroid Metabolism, Weight Loss & Reproductive Function

The possibility exists that there is a relationship between energy availability, thyroid metabolism, body weight loss and reproductive function. For example, the same threshold (between 11.4 and 18.1 kcal·kg FFM^{-1}·day^{-1}) that appears to have occurred in weight loss was identified for rT_3. Perhaps going below this threshold, constitutes a greater energy deficiency and the body can no longer maintain body weight and possible reproductive function (Beitins et al., 1991; Bullen et al., 1984). Similarly, in an effort for the physically active woman to lose body weight, through decreased caloric intake and/or an increase in energy expenditure, experimental results suggest that there comes a point at which the energy deficiency is too severe and irregularities in the menstrual cycles ensues (De Souza et al., 1998; Loucks et al., 1994b; Loucks et al., 1994c; Loucks et al., 1995; Williams et al., 1995; Loucks & Heath, 1998a; Loucks et al., 1998b).

The fact that the majority of studies find no significant difference in body weight between EUM and AMEN athletes suggests that reproductive function may be sacrificed as the body attempts to maintain weight. Moreover, though not significantly less, AMEN athletes tend to be 0.6-5.0 kg lighter than their EUM counterparts (Baer, 1993; Duester et al., 1986; Harber et al., 1998; Kaiserauer et al., 1989; Loucks et al., 1989; Perry et al., 1996; Snead et al., 1992; Wilmore et al., 1992). The AMEN athlete may be in a continual effort to reduce her body weight, and when making slight alterations in 'energy in' and/or 'energy out', she may go below the threshold for weight loss, rT_3 and reproductive function. There are few comparative results of rT_3 levels between AMEN and EUM athletes, as rT_3 is seldom analyzed. There are less convincing data that AMEN athletes may be below an rT_3 threshold, as AMEN athletes have significantly less rT_3 levels than their EUM counterparts (Loucks et al., 1992). One would expect an increase in rT_3 levels, to accompany low-T_3 levels induced by low energy availability. Furthermore, no differences in rT_3 levels were

found by Myerson et al. (1991) and Wilmore et al. (1992). However, Myerson et al. (1991) indicated that there was a trend of lower rT_3 levels in both AMEN and EUM runners. Moreover, as Loucks and Heath (1994a) point out, most AMEN athletes have practiced their dietary and exercise regimens for several years, and research has found that initially elevated rT_3 levels in obese individuals return to normal range when fasting for 2 weeks (Carlson et al., 1977) or dietary restriction (O'Brian et al., 1980) is prolonged for more than 3 weeks.

It may be possible that all three of these variables (ie. an attempt for weight loss, rT_3 levels and reproductive function) share a similar energy availability threshold or somehow influence each other. Even though there is usually no significant difference between body weight for AMEN and EUM athletes, perhaps AMEN athletes are trying more so to lose weight than EUM athletes (Baer, 1993; Myerson et al., 1991; Perry et al., 1996) as evidenced by significantly higher scores on the Eating Attitudes Test (EAT) (Myerson et al., 1991; Perry et al., 1996). Research has found that some physically active AMEN women consume significantly fewer calories (Baer, 1993; Kaiserauer et al., 1989; Marcus et al., 1985; Nelson et al., 1986) and have higher exercise energy expenditures (Baer, 1993; Drinkwater et al., 1984) compared to their EUM peers. Whereas, other studies have not found differences between AMEN vs EUM athletes caloric intakes and energy expenditures (Loucks et al., 1992; Myerson et al., 1991; Perry et al., 1996; Schwartz et al., 1981; Wilmore et al., 1992). The combined effect however, of slightly fewer dietary calories and a slightly higher caloric expenditure may put the AMEN athlete at a lower energy availability than her EUM counterpart. Perhaps AMEN athletes are just below and EUM athletes are just above the reproductive energy availability threshold, such that the EUM athletes are consuming just enough calories to maintain menstrual status, while their AMEN counterparts are not.

Subclinical and clinical alterations of the menstrual cycle were not reported after the completion of the present study. Monitoring the subsequent menstrual cycle with a questionnaire following the completion of the study would have been beneficial. For example, did the participant experience any menstrual cycle alterations (ie. longer/shorter/non-existent cycle, lighter/heavier flow etc.) ? It is possible that a correlation could have been identified, between the lower energy availability groups (ie. Groups 4 and 5) and an alteration in subjects' menstrual cycle following participation. Thus perhaps indicating the insult that the subjects' bodies had encountered during 4-days of low energy availability (during subjects early to late-follicular phase) which may have been enough to have a negative impact on reproductive function.

Effects of Energy Availability on Other Metabolic Indices

In addition to examining the effects of energy availability on thyroid metabolism in the present study, numerous other metabolic indices could have been examined. 'Lowered T_3 state' has been induced by 4-days of low energy availability (Loucks & Callister, 1993; Loucks & Heath, 1994a). Likewise, 4 or 5 days of low energy availability has resulted in reductions in insulin (Loucks & Verdun, 1998a; Loucks et al., 1998b), reductions in IGF-I (Loucks et al., 1998b), reductions in plasma glucose (Loucks & Verdun, 1998a; Loucks et al., 1998b), and increases in GH (Loucks et al., 1998b), increases in βhydroxybutyrate (Loucks & Verdun, 1998a) and increases in cortisol levels (Loucks et al., 1998b). The authors of these studies indicated that these changes occurred in their subjects (habitually sedentary EUM women) to mobilize body fat stores to supplement an inadequate dietary energy intake. Moreover, low energy availability has altered LH pulsatility so that there was a decrease in LH pulse frequency and an increase in LH pulse amplitude

(Loucks & Verdun, 1998a). Interestingly, similar LH pulsatility changes have been observed in physically trained EUM women with luteal suppression (Loucks et al., 1989).

Moreover, differences between AMEN and EUM physically trained women are revealed when examining metabolic hormones that regulate energy utilization and blood glucose levels. EUM and AMEN athletes both display suppressed insulin and elevated GH levels (Laughlin et al., 1994), but AMEN athletes also display low blood glucose levels (Laughlin et al., 1994), mildly elevated cortisol levels (De Souza et al., 1994; Dueck et al., 1996; Loucks et al., 1989; Villanueva et al., 1986), low T_3 and fT_3 levels (Loucks et al., 1992; Marcus et al., 1985), and high levels of insulin-like growth factor binding protein 1 (IGFBP-1) (Laughlin et al., 1994). As Loucks (1996) points out, because cortisol, like insulin, is a glucoregulatory hormone as well as a stress hormone, early interpretation of elevated cortisol levels in AMEN athletes was thought to be evidence of "exercise stress". However, these higher concentrations of cortisol may simply reflect a multifaceted endocrine response to energy deficiency. IGFBP-1 is a hepatic protein thought to modulate the growth-promoting actions of insulin-like growth factor 1 (IGF-1), which has been proposed as a signal to the hypothalamus and/or ovaries that helps integrate fuel availability and reproductive control (Jenkins et al., 1993).

Changes in metabolic indices induced by an acute bout of low energy availability in habitually sedentary women is reminiscent of cross-sectional observations of AMEN athletes. These findings suggest that AMEN athletes may be habitually performing at a lower energy availability (compared to EUM athletes) or some other metabolic change may be occurring (eg. reduced glucose availability to the brain, mobilization of free fatty acids) for the body to make requisite changes to deal with an energy deficient state.

Additional Factors for Consideration

The metabolic effects of varying energy availability is influenced by a variety of factors. An attempt must be made to control for these factors when examining this research area. One such factor is the difference between an individual's habitual energy intake and the number of calories consumed throughout the 4-days of controlled caloric intake. For example, Group 2 habitually consumed 300 to 400 less calories a day, compared to the other 4 groups (no significance between groups). Thus, when examining the difference in the amount of calories the subjects were used to consuming to their 4-day diet during the study, it appears as though Group 1 and 2 experienced the same degree of change in their diet (ie. they consumed ~700 kcal·day^{-1} more than they were used to consuming). Therefore, if the subjects in Group 2 were used to consuming fewer calories (and possibly living at a reduced energy availability) on a regular basis compared to the other groups, then they may have responded differently to the treatment. Moreover, perhaps Group 2 is at the same habitual energy availability as the other groups, whereby not expending as many calories through habitual exercise. However, if $\dot{V}O_{2max}$ and VT are indicators of one's fitness level and these indicators share a relationship with the amount and type (ie. intensity) of exercise performed, then Group 2 appears to have performed the same amount of exercise compared to the other 4 groups.

Another factor that one should take into consideration when examining the effects of energy availability on various hormones, is the time in which subjects start the 7-day study in relation to their menstrual cycle. Subjects who start the study later in their menstrual cycle may have a different hormonal profile compared to those who start the 7-day study earlier in their menstrual cycle. This may be of significance due to the fact that E_2 concentrations may have an influence on thyroid metabolism, whereby E_2 increases the peripheral conversion of T_4 to T_3 (Azizi, 1978). However, these differences only appear to be

found in obese women, who have significantly elevated E_2 levels (Azizi, 1978; Felig et al., 1995). Moreover, due to variations in menstrual cycle length, it would become very difficult to control for this variable (ie. subjects all starting and completing the study with similar E_2 concentrations).

The intensity level at which exercise is performed may also need to be controlled for when examining the metabolic effects of varying energy availability. Research to date indicates that exercising at intensities of 40% and 70% $\dot{V}O_{2max}$ does not influence changes in thyroid hormones (Loucks & Callister, 1993). Loucks & Callister (1993) note that the two different exercise intensities probably cause physiological processes to differ, including presumed differences in adrenal activation and presumed differences in proportions of glucose and fatty acids used as metabolic fuels, however, these differences appear to have no effect on thyroid metabolism. Nonetheless, it should be noted that Loucks & Callister (1993) only used two extreme energy availability groups set at 11.1 kcal·kg LBM^{-1}·day^{-1} (500 kcal·day^{-1}) and 38.7 kcal·kg LBM^{-1}·day^{-1}(1,750 kcal·day^{-1}). Thus, substrate utilization may have made a difference if these researchers were trying to identify thyroid thresholds, as CHO availability or free fatty acid mobilization may have been different due to the varying exercise intensities. Unfortunately, Loucks & Callister (1993) did not state RER values for the two exercise intensity groups and therefore one can not calculate the differences in substrate utilization and thus CHO availability to the brain or the degree of free fatty acid mobilization. Moreover, when determining the amount of energy expended during exercise, the RER values should be used, instead of assuming that 1 L of O_2 consumed is equivalent to 4.83 kcal (this equates to an RER of 0.82). This assumption is often made due to the small (4.9%) difference that exists between an RER of 0.80 and an RER of 1.00 (4.801 kcal·L^{-1} vs 5.047 kcal·L^{-1}), respectively. However, in study in which exercise is preformed for up to 4 hours, this assumed small difference could become a large difference (ie. ~100 kcal·hr^{-1}) and the determination of energy availability would be inaccurate.

Conclusion

The entire study group experienced 'lowered T_3 state' (ie. main effect of time for T_3, fT_3 and rT_3) and suggests that the physically trained woman may be more susceptible to the induction of 'lowered T_3 state' across the varying energy availabilities than the sedentary woman, whereby 'lowered T_3 state' is induced only at low energy availabilities (Loucks & Heath, 1994a). One thyroid threshold (ie. rT_3) was found for the physically trained woman, which occurred at a similar energy availability level as Loucks & Heath (1994a). Thus, it appears that regardless of a woman's training state or fitness level, lower energy availabilities appear to influence rT_3 in a similar fashion. There also appears to be a relationship between energy availability and body weight loss, insomuch that there may be a body weight loss threshold at the same energy availability threshold as that found for rT_3.

The effects of energy availability on substrate utilization indicated that regardless of the energy availability group, RER values were highest on the first day of exercise, during the first bout of exercise, whereas RER values were the lowest on the last day of exercise, during the last bout of exercise. Furthermore, the highest energy availability group (ie. Group 1) displayed the highest mean RER values, whereas the lowest energy availability group (ie. Group 5) displayed the lowest mean RER values compared to groups 1, 2 and 3. These findings indicate that there is a greater demand on fat stores as the level of energy availability decreases and that as the study progressed, the subjects CHO stores were progressively being depleted. Moreover, it appears that the physically trained woman has a higher degree of fat utilization compared to her sedentary counterpart, which may potentially enhance glucose availability to the brain, whereby being protective in nature in maintaining reproductive function.

Recommendations & Future Research

Factors which may have made it difficult to identify other thresholds (ie. T_3, fT_3 and T_4) include implementing a small sample size per energy availability group, and that the physically active women appeared to be accustomed to a reduced energy availability. It may be more difficult to identify thresholds in the physically trained EUM woman, as she may be just above, at, or even below a certain thyroid threshold. One must ensure that a higher level of energy availability is created, whereby those women who are below a threshold, may display hormonal changes by increasing their energy availability.

The AMEN athlete may have surpassed all thyroid thresholds, due to the suggestion that rT_3 (which appears to be the lowest thyroid threshold) and the reproductive threshold (ie. which causes complete menstrual function shut down once below it) may share the same level of energy availability. If AMEN athletes have surpassed all thyroid thresholds, the identification of increases in fT_3 and T_3 may evolve. Therefore, one may be able to distinguish if the AMEN athlete has similar thyroid thresholds identified in physically trained non-obese women, habitually sedentary non-obese women and habitually sedentary obese individuals. A study with AMEN athletes could be designed to see the effects of varying energy availabilities on metabolic and reproductive markers. Such a study however, would manipulate the AMEN athletes diet and exercise routine and it may be difficult to recruit subjects who would comply with having to consume more calories and/or exercise less to create energy availabilities above the ones' that they are currently living at. As suggested by Dueck et al. (1996), this may especially be the case in those AMEN athletes who have a major body weight concern or a distorted body image.

The various activities of Type I, Type II and Type III enzymes responsible for the conversion of thyroid hormones may account for 'lowered T_3 state'. It may be possible that depending on the physiological state of a certain organ (eg. liver, brain), these organs are able to regulate the activities of

thyroid metabolism and/or send messages to other organs (eg. thyroid) to regulate the activities of thyroid metabolism. For example, the liver (during ketosis), and the brain (during low glucose availability), may be major players in regulating thyroid metabolism. As well, both Type I and Type III enzymes appear to have a more global effect on thyroid metabolism, whereas Type II enzymes appear to reside mostly within organs (Leonard & Koehrle, 1996). Perhaps the activities of the Type II enzymes are the initial regulators in thyroid metabolism, which may subsequently influence Type I and Type III enzymes, or perhaps Type I and Type III enzymes are sensitive to 'whole body' changes such as a decrease in glucose, increase in ketones, GH, cortisol, IGF-I and insulin.

Further work needs to be performed to identify the mechanisms involved which induce 'lowered T_3 state' in the acute stages of low energy availability in the physically active EUM woman. Such work may permit more precise recommendations for the physically active woman who may put her health at risk in striving for the "ideal" body, whereby she lives at a reduced energy availability through the combination of restricting her dietary calories and expends a great amount of energy through exercise.

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APPENDIX A

RECRUITMENT POSTER

PHYSICALLY ACTIVE WOMEN !!!

Are you interested in learning your:

- ✔ **Daily nutritional intake**
- ✔ **Percent body fat**
- ✔ **Aerobic & anaerobic fitness levels (ie. VO_{2max}, anaerobic threshold)**

Researchers at the University of Alberta are examining the effects of exercise and caloric intake on metabolism in physically active women. You qualify for this research project if you answer "*YES*" to the following:

- ♀ 18 to 29 years of age
- ♀ Menstrual cycles 26-34 days in length
- ♀ No oral contraceptive pill use
- ♀ Stable body weight (± 3 kg in last 6 months)
- ♀ Exercise at least 4 hours a week

☞ VOLUNTEERS WILL BE INDIVIDUALLY MONITORED AND PROVIDED WITH FEEDBACK OF THEIR TEST RESULTS.

☞ TOTAL TIME COMMITMENT SHOULD NOT EXCEED 28 HOURS.

IF YOUR ARE INTERESTED IN FURTHER INFORMATION, *please* CONTACT:

SHERI FOSTER
WOMEN'S HEALTH & PHYSICAL ACTIVITY LABORATORY
FACULTY OF PHYSICAL EDUCATION & RECREATION
492-8739 OR SLFOSTER@GPU.SRV.UALBERTA.CA

Sheri Foster (slfoster@gpu.srv.ualberta.ca)
Women's Health & Physical Activity Lab
Faculty of Phys Ed & Rec @ 492-8739

APPENDIX B

STUDY INFORMATION SHEET

UNIVERSITY OF ALBERTA
FACULTY OF PHYSICAL EDUCATION AND RECREATION

Hormonal response to varying energy availabilities in physically active women

INVESTIGATORS: Dr. Vicki Harber, Sheri L. Foster, Dr. Gordon Bell, Dr. David Cumming

STUDY INFORMATION SHEET

PURPOSE

Inadequate energy availability, produced by increased energy expenditure and/or caloric restriction, alters a variety of metabolic processes throughout the body. Adaptations to low levels of energy might include reduced metabolic rate and difficulty with weight control.

A level of total energy availability has been identified in sedentary women, below which thyroid hormones (major regulators of metabolic rate) drop. Similar results are not available in physically active women but may be different from their sedentary counterparts. This study aims to determine a level of total energy availability in physically active women. Identification of this energy availability level may provide practical nutritional and exercise prescription guidelines to prevent metabolic stresses the body experiences while deprived of adequate energy.

The purpose of our study is to investigate the effects of different energy availability levels on thyroid hormones compared to sedentary women.

STUDY PROTOCOL

Fifty healthy, physically active women who are not using oral contraceptive pills, aged 18-35 will be recruited for this study and asked to complete the following:

I. ENTRY CRITERIA - These tests will be scheduled and conducted within 3 months prior to the start of the study.

1) Menstrual cycle tracking and health status questionnaires: Subjects will record their first day of menstruation for 3 consecutive months. A questionnaire will be completed to establish medical history, use of medications, menstrual history, and physical activity history. **(TIME= 1 HOUR)**

2) Aerobic fitness assessment: Maximal aerobic consumption (VO_2max) will be determined through progressive exercise to volitional fatigue on a cycle ergometer. Muscle discomfort/soreness, shortness of breath, and abnormal heart beat and blood

pressure are possible side effects associated with maximal aerobic consumption (VO_2max) but are rare in healthy young individuals. **(TIME = 1 HOUR)**

3) Body Composition: Body composition will be assessed from the sum of 9 skinfolds (measured with skinfold calipers at the biceps, triceps, subscapular, iliac crest, medial calf, front and rear thigh, abdominal, and midaxillary sites) and body density by underwater weighing. These tests are performed adjacent to the Women's Health Lab at the University of Alberta. A swimsuit is worn and the water is pleasantly warm. Changing facilities are located near the test pool. Body composition assessments will be done during days 1 through 6 of each subject's menstrual cycle. **(TIME = 1 ½ HOURS)**

4) Diet Records: Two diet records (3-Day and 7-Day) will be completed by the subjects prior to treatment. This will be analyzed on a computer software program to determine each subject's usual caloric intakes. **(TIME = 5 HOURS)**

II. 7-DAY TREATMENT - An attached diagram provides an overview of this project. All 7 days are consecutive and occur in the early follicular phase of the menstrual cycle (day 1 to day 9 of the cycle).

1) Blood Samples: A single blood sample (20 ml) will be taken by a physician, a registered nurse, or a trained phlebotomist every morning (6:30-9:30 a.m.) for the 7 test days. This will determine both baseline levels for estradiol and thyroid as well as adaptations to treatment. Bruising and a small risk of infection are possible (but rare) side effects associated with the acute venipunctures. **(TIME = 3 ½ HOURS)**

2) Exercise Prescription: On Test Days 3, 4, 5 and 6, subjects will exercise for a period of time equating a pre-set energy expenditure. This will approximate 2½-4 hours of exercise. Exercise will be performed for 30-minute intervals on a stationary cycle ergometer, with 10-minute rest intervals between each bout of exercise. Expired gases will be checked periodically to ensure appropriate intensity is maintained. No other strenuous exercise is permitted during these 4 days. **(TIME = 16 HOURS)**

3) Caloric Intake Prescription: During the 4 days of treatment (Test Days 3, 4, 5 and 6), total caloric intake will be provided by the researchers. Subjects will be randomly assigned to 1 of 5 experimental groups. Water may be consumed at any time. Each individual will be informed of their specific caloric assignment following the completion of the study.

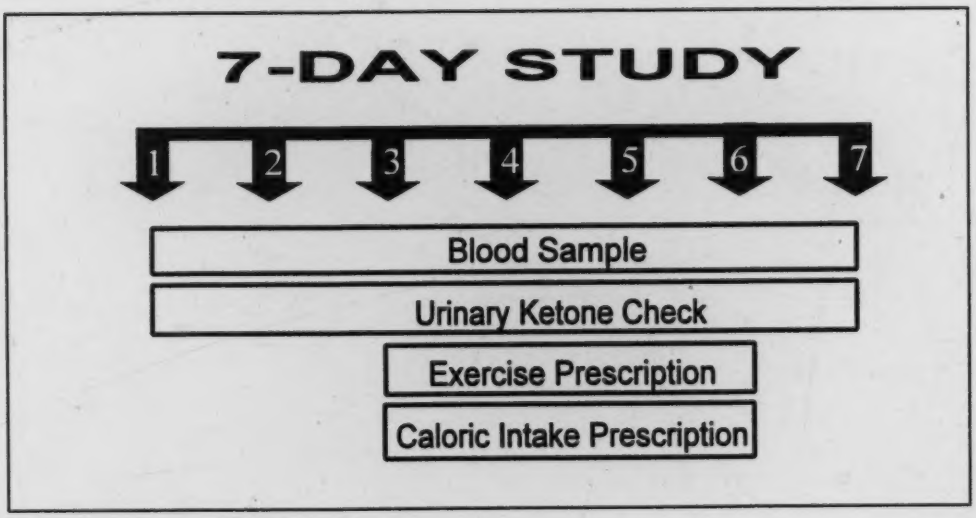
4) Urine Ketone Check: A small urine sample (10 ml) will be collected each morning (6:30-9:30 a.m.) for the 7 treatment days (Test Days 1 through 7) and analyzed for ketone acetoacetic acid levels. This identifies each volunteers' compliance regarding prescribed caloric intake and exercise levels during the study.

Total time commitment per subject would approximate 28 hours

CONFIDENTIALITY

The confidentiality of all data and subjects' identities will be ensured. All data will be locked in an office to which only the principle investigators will have access.

We strongly encourage questions for clarity and understanding of the above outlined experiment.
For further information, please feel free to contact
Sheri Foster @ 492-8739 (e-mail: slfoster@gpu.srv.ualberta.ca) or
Dr. Vicki Harber @ 492-1023 (e-mail: vharber@per.ualberta.ca)



APPENDIX C

SUBJECT CONSENT FORM

UNIVERSITY OF ALBERTA
FACULTY OF PHYSICAL EDUCATION AND RECREATION

Hormonal response to varying energy availabilities in physically active women

INVESTIGATORS: Dr. Vicki Harber, Sheri L. Foster, Dr. Gordon Bell, Dr. David Cumming

SUBJECT CONSENT FORM

This study has been satisfactorily explained to me by Dr. Vicki Harber, Sheri Foster, or their designate. I understand the necessity for the protocol outlined in the Study Information Sheet. I know that I may contact the persons designated on this form at any time if I have any further questions. I have been informed of the possible benefits of joining this research study as well as the possible risks and discomforts. I understand that there will be no costs to me for study-related visits. I have been assured that the information obtained from my participation in this study may be published in medical reports, but that my personal records will be kept confidential. I understand that I am free to withdraw from this study at any time without prejudice. I understand that I will be promptly informed of any findings which may develop during the research period that may affect my willingness to continue participating in the study. I understand that I will be given a copy of the Study Information Sheet and the signed Consent Form to keep.

Subject Name (print)	Subject Signature & Date
Witness Name (print)	Witness Signature & Date
Investigator Name (print)	Investigator Signature & Date

Questions or concerns may be directed to
Sheri Foster @ 492-8739 (E-mail: slfoster@gpu.srv.ualberta.ca) or
Dr. Vicki Harber @ 492-1023 (E-mail:vharber@per.ualberta.ca)

APPENDIX D

AEROBIC FITNESS TEST

AEROBIC FITNESS TEST

Data Sheet - Monark Bike

Name: _____ Group: _____

Date: _____ Age: _____ Weight (kg): _____

TIME (minutes)	RESISTANCE (kg)	REVOLUTIONS (rpm)	HEART RATE (bpm)	
0:00 - 1:00	1.0			
1:00 - 2:00	1.0			
2:00 - 3:00	1.5			
3:00 - 4:00	1.5			
4:00 - 5:00	2.0			
5:00 - 6:00	2.0			
6:00 - 7:00				
7:00 - 8:00				
8:00 - 9:00				
9:00 - 10:00				
10:00 - 11:00				
11:00 - 12:00				
12:00 - 13:00				
13:00 - 14:00				

TEST RESULTS:

Max HR: _____(bpm) $VO_{2\,max}$: _____ ($L \cdot min^{-1}$); _____ ($ml \cdot kg^{-1} \cdot min^{-1}$)

VT: _____($L \cdot min^{-1}$); _____ ($ml \cdot kg^{-1} \cdot min^{-1}$) VT (% of $VO_{2\,max}$): _____ (%)

HR @ VT: _____ (bpm) Workload @ VT: _____ (kg) RPM's @ VT: _____(rpm)

90% of VT: _____ ($L \cdot min^{-1}$); _____($ml \cdot kg^{-1} \cdot min^{-1}$) 90% of VT (% of $VO_{2\,max}$): ____(%)

APPENDIX E

DEMOGRAPHIC QUESTIONNAIRE

DEMOGRAPHIC QUESTIONNAIRE

Name:_____ Date:_____

Address: _____ Telephone:_____(Home)

_____ ___ _____(Work)

_____ E-mail: _____

Date of Birth:_____ Age:_____

Answer the following questions as accurately as possible.
Please ask for clarification where needed.

1. Have you experienced a weight loss or gain (±3 kg =±6.6 lbs or more) in the last 6months ?

YES_____ NO_____

If yes, specify the amount of weight lost (-) or gained (+) _____

2. Have you ever been diagnosed with an eating disorder ? YES_____ NO_____

3. List any prescribed medication or over-the-counter medication you regularly take:

_____

_____

_____

4. Do you have a heart, liver, or renal disease ? YES_____ NO_____

5. Do you have diabetes or a thyroid disorder ? YES_____ NO_____

6. Do you have any chronic or "nagging" musculoskeletal aches or pains (eg. sore knees, weak back)? YES_____ NO_____

If yes, indicate the location of your ache or pain and describe any related physical limitations.

_____

_____

7. Are you allergic to any drugs, foods or beverages? YES_____ NO_____

If yes, please list: _____

_____

8. Do you smoke? YES_____ NO_____

MENSTRUAL CYCLE

1. At what age did you have your first menstrual period? _____ (years)

2. Have you taken oral contraceptive pills within the last 6 months? YES_____ NO_____

3. Is your menstrual cycle regular (ie. every 24-35 days)? YES_____ NO_____

☞ "NO" (answer the next 4 questions then go to "Physical Activity" questions).

(i) When was the last time you menstruated? _____

(ii) How many periods do you usually have in a year? _____

(iii) On average, how many days does your period last? _____

(iv) What is the longest time you have gone without a period? _____

MENSTRUAL CYCLE cont'd

☞ "YES" (answer the next 4 questions then go to "Physical Activity" questions).

(i) How many periods do you usually have in a year ? _____

(ii) On average, how many days does your period last ? _____

(iii) What is the interval of days between your periods ? Indicate the number of days between "Day 1" (onset of flow) of a period, and "Day 1" of the next period _____

(iv) When was the last time you menstruated ? _____

If known, please indicate the last three "Day 1's" of your menstrual cycle:

_____

_____

_____

PHYSICAL ACTIVITY

1. Are you involved in a regular routine of physical activity ? YES_____ NO_____

☞ If "YES" . . .

(a) Does your routine include **4 OR MORE SESSIONS** a week ? YES___NO___

☞ How long have you been doing this routine for ? _____

(b) Does your routine **EXCEED 3 HOURS A WEEK** ? YES____ NO____

☞ How long have you been doing this routine for ? _____

3. Please list and describe ALL of your physical activities that you are involved in:

ACTIVITY	DURATION (min/session)	FREQUENCY (sessions/week)	INTENSITY*
eg. Running	45	5	1 2 3 4 5
1.			1 2 3 4 5
2.			1 2 3 4 5
3.			1 2 3 4 5
4.			1 2 3 4 5
5.			1 2 3 4 5
6.			1 2 3 4 5

* INTENSITY: 1 - Not vigorous at all (very light) 2 - Somewhat vigorous (light)
3 - Moderately vigorous (medium) 4 - Vigorous (heavy) 5 - Extremely vigorous (very heavy)

4. Do you belong to a sports team/club ? NO_____ (Please go to the next page)

YES_____(Please answer the following questions)

5. Name of team/club: _____

6. Sport: _____

7. Number of years competing in the sport:_____(years)

8. What is the highest level of competition that you have been involved in ?

Intramurals _____ City _____ Provincial _____ Varsity _____

National _____ International _____ Other (please specify) _____

9. Please feel free to add other comments with respect to your involvement in sports:

SUBJECT AVAILABILITY

All tests and exercise sessions will be scheduled at your convenience. One test (ie. body composition) and the 7-day experiment will require coordination with your menstrual cycle. Please indicate (with a "✔") the times for each day when you are available for testing and exercise sessions.

Time Slot	Mon	Tues	Wed	Thurs	Fri	Sat	Sun
7A.M.- 8A.M.							
8A.M. - 9A.M.							
9A.M. - 10A.M.							
10A.M.-11A.M.							
11A.M.-Noon							
Noon - 1P.M.							
1P.M.- 2P.M.							
2P.M.- 3P.M.							
3P.M.- 4P.M.							
4P.M.- 5P.M.							
5P.M.- 6P.M.							
6P.M.- 7P.M.							
7P.M.- 8P.M.							
8P.M.- 9P.M.							
9P.M.- 10P.M.							

Please feel free to add other comments you think are important for us to know: _____

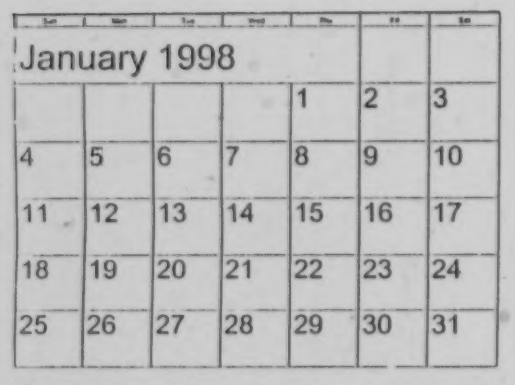
_____

THANK-YOU ! ! !

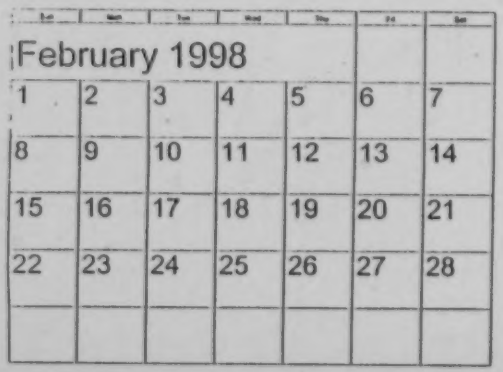
APPENDIX F

MENSTRUAL TRACKING CALENDARS

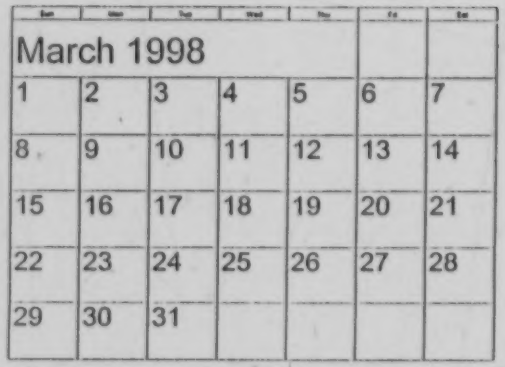
MENSTRUAL TRACKING CALENDARS



January 1998						
				1	2	3
4	5	6	7	8	9	10
11	12	13	14	15	16	17
18	19	20	21	22	23	24
25	26	27	28	29	30	31



February 1998						
1	2	3	4	5	6	7
8	9	10	11	12	13	14
15	16	17	18	19	20	21
22	23	24	25	26	27	28



March 1998						
1	2	3	4	5	6	7
8	9	10	11	12	13	14
15	16	17	18	19	20	21
22	23	24	25	26	27	28
29	30	31				

April 1998						
			1	2	3	4
5	6	7	8	9	10	11
12	13	14	15	16	17	18
19	20	21	22	23	24	25
26	27	28	29	30		

❀ You have received a series of calendars to keep track of your menstrual cycle for 3 consecutive months.

❀ Place an "X" on the date that corresponds with your *first day of bleeding* (ie. "Day 1").

❀ Indicate on the reverse side, if you experience irregular symptoms.

❀ Please call us for clarification if you are uncertain of anything.

Helpful Tips:

☛ Keep your calendars somewhere visible - a reminder for yourself.

☛ Associate your "Day 1" with an event of that day. This will make it easier to recall if you have forgotten to record it.

MENSTRUAL TRACKING CALENDARS
Irregular Symptoms

❀ Please use the space below to record information regarding your menstrual cycles (ie. Irregular spotting/bleeding, heavy/light flow, length of menstruation, irregular symptoms...etc.).

❀ Please indicate if you are sick or have a fever at anytime throughout tracking your menstrual cycle.

CYCLE # 1:

CYCLE # 2:

CYCLE # 3:

APPENDIX G

WHAT DO YOU EAT ???
GUIDELINES FOR YOUR 3 & 7-DAY DIET RECORDS

WHAT DO YOU EAT ???

Guidelines for your 3 & 7-Day Diet Records

Please record your daily intake of food and fluids for 3 and 7 consecutive days. Your 3-day diet record must be a Thursday/Friday/Saturday or a Sunday/Monday/Tuesday combination. Your 7-day diet record may begin on any day. The starting dates for you to begin recording your **NORMAL** dietary intake will be given to you. These dietary records will be analysed and you will receive information on the amount of calories, protein, fat and carbohydrates that you consume.

It is imperative that you record **EVERYTHING** that you eat and drink (water as well !). In addition, you must be as **ACCURATE** as possible when determining the amount (volume or weight) of the food and drink you are recording. *Please* use measuring cups/spoons and weigh scales whenever possible.

HINTS FOR RECORDING DIETARY INTAKE

ACCURACY

1. **ACCURATE MEASUREMENT** Read the weights or volumes of foods or drinks from packages. Example: milk carton, juice box, chocolate bar, potato chips. A "fistful" of meat = 100 grams, "fistful" veggies = 1 cup, 1 cheese single = 1 oz.

2. **METHOD OF COOKING** Indicate how your food was cooked. Example: fried, steamed, baked, broiled etc.

3. **"EXTRAS"** Don't forget the extras. Example: ketchup, mustard, mayonnaise, gravy, or butter.

4. **FOOD TYPES** Be specific about types of food/drink. Example: cheddar cheese, 2% milk, margarine or butter. Whenever possible, identify brand names of the foods.

5. **COOKED OR DRY MEASUREMENT** Indicate whether the food measurement is "cooked" or "dry". Example: chicken weight before or after cooked.

6. **SPECIFIC PARTS** Indicate the exact part of the food you ate or what was removed before eating. Example: chicken (white or dark, bone in or out, skin or skinless), baked potato (skin or skinless), ground beef (lean, extra lean, or regular).

7. **LABELS** If possible, please include the nutritional information label from the container (box/can/bag). Instead of including the actual label information, you may wish to write this information on the last page of your diet record or put it on a "post-it-note".

BEVERAGES

8. **TEA** and **COFFEE** should be included as well as the cream, milk and sugar you add.

9. Don't forget **WATER !**

10. Yes, you do have to record **BEER** and **ALCOHOL** as well.....!!

PREPARED OR RESTAURANT MEALS

11. Use **PORTION PAKS** whenever possible. Example: salad dressing, butter, jams, peanut butter, cheese. It is easier to quantify the volume of these foods...1 portion pak = 1 tablespoon.

12. **FAST FOODS** Include fast food items by name. Example: McDonald's, Pizza Hut, Wendy's.

13. **RECIPES** Record the amount/volume of ingredients, the number of servings or volume the entire recipe makes and how many servings or what volume you ate.

14. **RESTAURANT MEALS** When you eat at a restaurant (other than a fast food place, eg. Earl's), record the name of the meal you ate, list the different ingredients on your plate and the quantities of each.

TAKE THE RECORD BOOK WITH YOU AT ALL TIMES . . .
IT'S EASIER TO RECORD WHAT YOU'RE EATING.

APPENDIX H

EXAMPLE DIET RECORD

	MENU ITEM	UNIT OF MEAS		DESCRIPTION OF MENU ITEM		
	Enter all foods, beverages, etc. consumed as menu items. For every menu item, include any toppings or additives added to the menu item at the time of eating	Enter the word "cup" "ounce" "number" "teaspoon" "tablespoon"	No. of Units	Brand	Type of Flavour	Method of Cooking
EVENING MEAL	Menu item					
	Toppings or Additives					
	Menu item					
	Toppings or Additives					
	Menu item					
	Toppings or Additives					
	Menu item					
	Toppings or Additives					
	Menu item					
	Toppings or Additives					
	Menu item					
	Toppings or Additives					
	Mark (X) One Category	Eaten at Your Home				
		Eaten Away From Your Home				
		Did Not Eat				

Day Three

	MENU ITEM	UNIT OF MEAS		DESCRIPTION OF MENU ITEM		
	Enter all foods, beverages, etc. consumed as menu items. For every menu item, include any toppings or additives added to the menu item at the time of eating	Enter the word "cup" "ounce" "number" "teaspoon" "tablespoon"	No. of Units	Brand	Type of Flavour	Method of Cooking
MORNING MEAL	Menu item: eggs	number	2	Fantasy		scrambled
	Toppings or Additives: Ketchup	tablespoon	2			
	Menu item: sausage links	number	6	Schneiders	sausage	fried
	Toppings or Additives					
	Menu item: whole wheat toast	cut	2	Silvercrest		
	Toppings or Additives: Rose salt	tablespoon	2			
	Menu item: corn flakes	cup	1	Kellogg	corn flakes	
	Toppings or Additives: whole milk	cup	1			
	Toppings or Additives: sugar	t.s.	1			
	Menu item: banana	no.	1			
	Toppings or Additives					
	Menu item: mixed vitamin	number	1	Centrum		
	Toppings or Additives					
	Mark (X) One Category: Eaten at Your Home					
	Eaten Away From Your Home					
	Did Not Eat					

Sample Day

APPENDIX I

DAILY NUTRITION RECOMMENDATIONS

DAILY NUTRITION RECOMMENDATIONS

PLEASE NOTE: When "Nutrient Information Labels/Values" are entered into the computer (ie. Calories, carbohydrates, protein & fat), some of the values (ie. fat-saturated, fiber, cholesterol, calcium, iron, alcohol, caffeine) are not included and therefore your nutrient values may actually be HIGHER than indicated. Please take this into consideration when reviewing your results.

MACRONUTRIENTS

✓ Carbohydrate

The recommended dietary intake of carbohydrate is approximately **60%** of total daily calories. This percentage can be increased for highly active individuals. Carbohydrates are starches and sugars that fuel your brain and are the primary energy source when you are exercising at a high intensity.

✓ Protein

The recommended dietary intake of protein is approximately **15%** of total daily calories. Protein is essential for building and repairing muscles, red blood cells and synthesizing hormones.

✓ Fat

The recommended dietary intake of fat is **30%** or less of total daily calories. Fat is essential for normal cell function. It is needed to synthesize hormones, to insulate nervous tissue, provide cushioning for internal organs and offers a layer of protection against the cold.

TYPES OF FAT

1. Saturated Fat - Recommended daily intake is no more than 10% of total daily calories from butter or animal fats, or from coconut and palm oils - two highly saturated vegetable oils that are commonly used in processed foods.

2. Mono-Unsaturated Fat - Recommended daily intake is at least 10 % of total daily calories and is found most often in plant sources (eg. olive & canola oil).

3. Poly-Unsaturated Fat - Recommended daily intake is about 10% of total daily calories and is found mainly in plant sources (eg. corn & safflower oil).

DIETARY FIBER

The recommended daily intake of dietary fiber is 25 - 35 grams per day. Dietary fiber is the part of plant cells in food that humans cannot digest. It promotes regularity, lowers blood cholesterol, improves blood sugar control, and may even protect against colon cancer. Some sources of dietary fiber are fruits, vegetables, and whole grain foods.

ALCOHOL

Alcohol contributes calories to your diet, but these are nutrient deficient. Moreover, alcohol is a diuretic and therefore should only be consumed in moderation with plenty of water.

CAFFEINE

Caffeine is a naturally occurring compound in many foods and beverages that we consume everyday. It is believed that 230 - 460 milligrams may enhance sport performance, however, high levels may negatively affect performance (>1300 milligrams).
NOTE: 10- ounces or 1 1/4 cups of coffee has approximately 150-230 milligrams of caffeine.
The consumption of caffeine to benefit performance is an extremely individual matter. For some it is key to happiness, while for others it gives them the jitters and stomach turmoil. Caffeine is a diuretic and therefore should be compensated for by drinking plenty of water.

WATER AND MINERALS

➢ Water

The recommended intake of water is 2 litres (8 cups) per day. Your requirements will increase with exercise and/or hot temperatures and humidity. Water is crucial for the regulation body temperature, carriage of nutrients to and waste away from cells, and is needed for proper cell functioning.

➢ Iron

It is recommended that women consume 15-18 milligrams of iron per day. Iron is a necessary component of hemoglobin, the protein that transports oxygen throughout the body. Thus, a deficiency in iron will cause fatigue upon physical exertion. **The best sources of iron are lean cuts of red meats and poultry, and fortified breads and cereals. To enhance absorption of iron, eat iron-rich foods with vitamin C and avoid consuming them with caffeinated foods.**

➢ Calcium

Recommended daily intake for an adult is 1000 milligrams of calcium per day, or 2 - 4 servings per day (for serving suggestions and more information on calcium, please review the "Calcium for Life" pamphlet).

CHOLESTEROL

This substance contributes to hardening of the arteries as it accumulates in the walls of the blood vessels throughout the body, especially in the heart. This can lead to heart attacks. For most athletes, high cholesterol is not a concern, however, it is still valuable to know that the recommended dietary intake is less than 300 milligrams per day.

Additional Readings:

Clark, N. 1997. *Nancy Clark's Sports Nutrition Guidebook, 2nd.* Champaign, IL. Human Kinetics.

Dairy Nutrition Council of Alberta. 1997.
Sport Nutrition: Tips on food intake and fluid replacement to help athletes gain the "winning edge" in competition !

APPENDIX J

BODY COMPOSITION ANALYSIS

BODY COMPOSITION ANALYSIS

Data Sheet

Name: _____ Group: _____

Date: _____ Day of Cycle: _____ Age: _____

Tester(s): _____

Height (cm): _____ Weight (kg): _____

SKINFOLDS (mm)				
LANDMARK	TRIAL 1	TRIAL 2	TRIAL 3	AVERAGE
TRICEPS				
BICEPS				
SUBSCAPULAR				
MIDAXILLARY				
ILIAC CREST				
ABDOMINAL				
FRONT THIGH				
REAR THIGH				
MEDIAL CALF				

HYDROSTATIC WEIGHING		
	PREDICTED (age, height)	ACTUAL
RESIDUAL VOLUME (L)		
BODY FAT (%)		
FAT BODY MASS (kg)		
LEAN BODY MASS (kg)		

APPENDIX K

7-DAY TREATMENT URINE ANALYSIS DATA SHEET

7-DAY TREATMENT

Urine Analysis Data Sheet

NAME: _____ GROUP: _____ # of Cans: _____

DAY #1 - DATE: _____ Time: _____ Analyzer: _____

QUESTION	ANSWER
Have you fasted since midnight ?	
Did you consume your usual amount of food & beverages yesterday ? (If no, please explain)	
Did you exercise yesterday ? (If yes, what did you do & is this a usual workout ? If not your usual workout, please explain)	
Colour of keto-stix ?	

Concerns/Comments:

DAY #2 - DATE: _____ Time: _____ Analyzer: _____

QUESTION	ANSWER
Have you fasted since midnight ?	
Did you consume your usual amount of food & beverages yesterday ? (If no, please explain)	
Did you exercise yesterday ? (If yes, what did you do & is this a usual workout ? If not your usual workout, please explain)	
Colour of keto-stix ?	

Concerns/Comments:

DAY #3 - DATE: _____ Time: _____ Analyzer: _____

QUESTION	ANSWER
Have you fasted since midnight ?	
Did you consume your usual amount of food & beverages yesterday ? (If no, please explain)	
Did you exercise yesterday ? (If yes, what did you do & is this a usual workout ? If not your usual workout, please explain)	
Colour of keto-stix ?	

7-DAY TREATMENT

Urine Analysis Data Sheet

NAME: _____ GROUP: _____ # of Cans: _____

	Day #4	Day #5	Day #6	Day #7
	Compliant "✓"	Compliant "✓"	Compliant "✓"	Compliant "✓"
Date				
Time				
Analyzer				
Have you fasted since midnight ?				
Did you consume all of your liquid nutritional supplement yesterday ?				
Did you consume anything else besides your liquid nutritional supplement yesterday ?				
Did you exercise outside of the lab yesterday ? (If yes, what did you do ?)				
Colour of keto-stix ?				

Concerns/Comments:

APPENDIX L

7-DAY TREATMENT EXERCISE DATA SHEET

start time: _____

finish time: _____

7-DAY TREATMENT
Exercise Data Sheet

intensity: _____

range: _____

NAME: _____ GROUP: _____ Number of Cans: _____

Workload @ 90% VT: _____ (kg) RPM's @ 90% VT: _____ (rpm) Duration Required @ 90% VT (min): _____ (hr:min): _____

DAY # ___ - DATE: _____ Supervisor(s): _____

"✓" (when completed)	Heart Rate / Resistance / Revolutions per minute (every 5 min)	Metabolic Cart Print-Out
❑ 1st 30 min of Exercise	5 min:____ 10 min:____ 15 min:____ 20 min:____ 25 min:____ 30 min:____	❑
❑ 1st 10 min of Rest		
❑ 2nd 30 min of Exercise	5 min:____ 10 min:____ 15 min:____ 20 min:____ 25 min:____ 30 min:____	❑
❑ 2nd 10 min of Rest		
❑ 3rd 30 min of Exercise	5 min:____ 10 min:____ 15 min:____ 20 min:____ 25 min:____ 30 min:____	❑
❑ 3rd 10 min of Rest		
❑ 4th 30 min of Exercise	5 min:____ 10 min:____ 15 min:____ 20 min:____ 25 min:____ 30 min:____	❑
❑ 4th 10 min of Rest		
❑ 5th 30 min of Exercise	5 min:____ 10 min:____ 15 min:____ 20 min:____ 25 min:____ 30 min:____	❑
❑ 5th 10 min of Rest		
❑ 6th 30 min of Exercise	5 min:____ 10 min:____ 15 min:____ 20 min:____ 25 min:____ 30 min:____	❑

APPENDIX M

STUDY DATA FOR ALL SUBJECTS

ID	Group	Pre Tot-T3	Post Tot-T3	Diff Tot-T3	%Diff Tot-T3	Pre Tot-T4	Post Tot-T4	Diff Tot-T4	%Diff Tot-T4	Pre free-T3
11	1	99.45	90.98	-8.47	-8.52	5.92	7.22	1.30	21.96	2.670
12	1	96.25	106.79	10.54	10.95	7.03	8.30	1.27	18.07	2.750
13	1	111.37	92.45	-18.92	-16.99	6.20	4.85	-1.35	-21.77	3.551
14	1	61.90	51.31	-10.59	-17.11	3.55	4.00	0.45	12.68	1.735
15	1	110.39	118.47	8.08	7.32	6.36	6.84	0.48	7.55	3.451
21	2	111.10	94.87	-16.23	-14.61	5.46	5.45	-0.01	-0.18	3.352
22	2	96.22	88.64	-7.58	-7.88	6.30	6.26	-0.04	-0.63	2.858
23	2	89.59	80.31	-9.28	-10.36	6.97	6.47	-0.50	-7.17	2.425
24	2	115.10	97.98	-17.12	-14.87	5.30	4.55	-0.75	-14.15	3.729
25	2	86.99	90.27	3.28	3.77	3.83	4.09	0.26	6.79	2.187
26	2	86.09	81.71	-4.38	-5.09	3.84	3.84	0.00	0.00	2.239
31	3	114.47	101.55	-12.92	-11.29	7.49	6.74	-0.75	-10.01	2.754
32	3	95.50	79.88	-15.62	-16.36	5.20	4.63	-0.57	-10.96	2.760
33	3	100.43	95.18	-5.25	-5.23	6.28	6.54	0.26	4.14	2.478
34	3	103.41	82.33	-21.08	-20.38	4.90	5.04	0.14	2.86	2.946
35	3	79.29	87.37	8.08	10.19	4.79	5.09	0.30	6.26	2.651
36	3	106.04	103.68	-2.36	-2.23	7.56	7.60	0.04	0.53	3.126
41	4	119.55	111.21	-8.34	-6.98	7.20	7.14	-0.06	-0.83	3.607
42	4	85.36	109.98	24.62	28.84	4.51	4.63	0.12	2.66	2.163
43	4	84.44	77.33	-7.11	-8.42	3.90	4.21	0.31	7.95	2.262
44	4	90.64	72.92	-17.72	-19.55	5.42	6.23	0.81	14.94	2.469
45	4	83.85	68.10	-15.75	-18.78	6.61	5.92	-0.69	-10.44	2.437
46	4	89.95	78.01	-11.94	-13.27	5.90	6.12	0.22	3.73	2.263
51	5	95.82	82.24	-13.58	-14.17	4.73	5.01	0.28	5.92	2.665
52	5	109.43	86.92	-22.51	-20.57	5.25	5.77	0.52	9.90	2.854
53	5	101.35	100.33	-1.02	-1.01	5.33	5.52	0.19	3.56	3.056
54	5	88.19	89.98	1.79	2.03	3.90	4.60	0.70	17.95	2.365
55	5	80.82	73.55	-7.27	-9.00	4.97	4.73	-0.24	-4.83	1.831
56	5	102.29	84.49	-17.80	-17.40	6.42	6.06	-0.36	-5.61	2.195

Post free-T3	Diff free-T3	%Diff free-T3	Pre free-T4	Post free-T4	Diff free-T4	%Diff free-T4	Pre rev-T3	Post rev-T3	Diff rev-T3
2.411	-0.259	-9.70	1.428	1.471	0.043	3.01	0.156	0.193	0.037
3.241	0.491	17.85	1.811	2.000	0.189	10.44	0.193	0.219	0.026
3.045	-0.506	-14.25	1.772	1.136	-0.636	-35.89	0.174	0.122	-0.052
1.844	0.109	6.28	1.017	1.052	0.035	3.44	0.097	0.123	0.026
3.507	0.056	1.62	1.670	1.660	-0.010	-0.60	0.176	0.145	-0.031
2.866	-0.486	-14.50	1.412	1.559	0.147	10.41	0.164	0.162	-0.002
2.761	-0.097	-3.39	1.641	1.531	-0.110	-6.70	0.124	0.143	0.019
2.099	-0.326	-13.44	1.730	1.625	-0.105	-6.07	0.239	0.257	0.018
3.029	-0.700	-18.77	1.351	1.377	0.026	1.92	0.157	0.160	0.003
2.418	0.231	10.56	1.014	1.137	0.123	12.13	0.097	0.123	0.026
2.541	0.302	13.49	1.035	0.948	-0.087	-8.41	0.099	0.091	-0.008
2.499	-0.255	-9.26	1.845	1.594	-0.251	-13.60	0.232	0.208	-0.024
2.004	-0.756	-27.39	1.339	1.210	-0.129	-9.63	0.135	0.145	0.010
2.452	-0.026	-1.05	1.515	1.389	-0.126	-8.32	0.227	0.247	0.020
2.165	-0.781	-26.51	1.054	1.155	0.101	9.58	0.147	0.146	-0.001
2.748	0.097	3.66	1.166	1.478	0.312	26.76	0.127	0.138	0.011
2.965	-0.161	-5.15	1.791	1.713	-0.078	-4.36	0.202	0.201	-0.001
2.799	-0.808	-22.40	1.496	1.548	0.052	3.48	0.155	0.182	0.027
2.367	0.204	9.43	0.831	1.154	0.323	38.87	0.078	0.131	0.053
2.321	0.059	2.61	0.752	0.847	0.095	12.63	0.066	0.101	0.035
2.082	-0.387	-15.67	1.236	1.317	0.081	6.55	0.149	0.180	0.031
1.883	-0.554	-22.73	1.630	1.437	-0.193	-11.84	0.218	0.234	0.016
1.887	-0.376	-16.62	1.348	1.378	0.030	2.23	0.124	0.147	0.023
2.334	-0.331	-12.42	1.035	1.182	0.147	14.20	0.117	0.143	0.026
2.027	-0.827	-28.98	1.108	1.017	-0.091	-8.21	0.114	0.149	0.035
2.864	-0.192	-6.28	1.059	0.971	-0.088	-8.31	0.091	0.105	0.014
2.498	0.133	5.62	0.797	0.943	0.146	18.32	0.081	0.105	0.024
1.478	-0.353	-19.28	0.920	0.850	-0.070	-7.61	0.091	0.148	0.057
1.542	-0.653	-29.75	1.064	1.124	0.060	5.64	0.130	0.172	0.042

%Diff rev-T3	E2 - Day 1	E2 - Day 3	E2 - Day 7	actual energy expended (kcal/day)	actual energy expended (kcal/FFM/day)	actual 4-d diet (kcal/day)	actual 4-d diet (kcal/FFM/day)	average 7-d diet (kcal/day)
23.72	31.14	45.30	62.70	1162	31.84	2380	65.21	1809
13.47	72.91	90.24	77.71	1594	32.54	3445	70.32	2427
-29.89	50.71	49.96	59.11	1380	31.02	3130	70.34	1860
26.80	23.86	35.96	53.49	1458	31.41	3235	69.72	3190
-17.61	57.47	77.48	114.17	1612	31.37	3590	69.84	2987
-1.22	54.18	46.93	99.27	1541	30.88	2670	53.51	1465
15.32	67.40	91.07	189.76	1360	27.93	2670	54.83	2668
7.53	59.64	66.40	54.83	1618	33.51	2670	55.28	1643
1.91	97.22	82.95	54.37	1247	30.26	2250	54.61	2225
26.80	38.56	40.43	130.85	1686	29.63	3130	55.01	2003
-8.08	53.66	74.98	269.01	1393	26.89	2880	55.60	1960
-10.34	46.77	56.49	69.97	1367	32.40	2085	49.41	2724
7.41	25.59	38.70	40.08	1565	28.71	2670	48.99	1760
8.81	47.77	51.40	54.95	1614	31.64	2525	49.50	2909
-0.68	60.87	56.79	51.59	1490	33.04	2210	49.00	3048
8.66	36.32	43.85	85.78	1776	31.32	2775	48.94	2284
-0.50	43.48	51.03	60.21	1289	29.50	2170	49.66	1721
17.42	38.12	43.85	29.27	1293	29.38	1875	42.61	2016
67.95	29.71	35.94	46.26	1910	30.46	2525	40.27	2317
53.03	39.10	33.59	31.63	1350	29.28	1920	41.64	2605
20.81	37.32	45.12	45.48	1454	29.67	2000	40.82	2188
7.34	29.80	48.83	47.82	1752	32.03	2250	41.13	2316
18.55	51.53	53.48	150.17	1418	28.14	2065	40.97	2479
22.22	23.38	34.83	40.13	1406	30.36	1625	35.10	2891
30.70	59.46	48.50	106.70	1221	28.19	1500	34.63	1656
15.38	42.70	41.23	60.00	1569	32.03	1710	34.92	2419
29.63	67.31	56.62	108.62	1577	29.75	1855	35.00	2305
62.64	41.20	43.96	34.30	1171	27.30	1500	34.97	1935
32.31	42.66	34.77	30.03	1436	28.66	1750	34.93	2750

average 7-d diet (kcal/FFM/day)	diff between 4-d & 7-d (kcal/day)	diff between 4-d & 7-d (kcal/FFM/day)	energy avail (kcal/day)	energy avail (kcal/FFM/day)	Age (yr)	Age @ Menarche (yr)	Gyne Age (yr)	Cycle Length (last one)	Cycle Length (2nd last one)	Cycle Length (3rd last one)
49.57	571	15.63	1218	33.36	24	14	10	33	43	34
49.55	1018	20.77	1851	37.78	23	13	10	22	33	
41.79	1270	28.54	1750	39.32	25	15	10	30	27	28
68.75	45	0.97	1777	38.31	32	11	21	30	29	29
58.12	603	11.73	1978	38.48	26	13	13	28	30	26
29.36	1205	24.15	1129	22.63	25	14	11	29	28	30
54.79	2	0.04	1310	26.90	32	15	17	23	23	27
34.01	1027	21.27	1052	21.77	18	14	4	27	26	23
54.02	25	0.60	1003	24.35	21	12	9	36	35	21
35.21	1127	19.80	1444	25.38	22	12	10	28	28	28
37.85	920	17.75	1487	28.71	30	12	18	25	28	25
64.54	-639	-15.13	718	17.00	25	12	13	37	31	29
32.29	910	16.70	1105	20.28	19	12	7	23	23	29
57.02	-384	-7.52	911	17.86	21	14	7	29	29	29
67.58	-838	-18.58	720	15.96	19	13	6	28	34	29
40.28	491	8.66	999	17.62	33	13	20	35	28	28
39.38	449	10.27	881	20.15	19	12	7	31	31	31
45.82	-141	-3.20	582	13.23	19	13	6	30	31	32
36.95	208	3.32	615	9.81	22	13	9	39	31	31
56.49	-685	-14.85	570	12.36	24	12	12	23	30	25
44.66	-188	-3.84	546	11.15	22	13	9	25	26	28
42.34	-66	-1.21	498	9.11	20	11	9	35	26	30
49.18	-414	-8.21	647	12.84	23	12	11	27	26	35
62.43	-1266	-27.33	219	4.74	17	14	3	28	28	26
38.24	-156	-3.60	279	6.45	24	14	10	26	25	27
49.39	-709	-14.47	141	2.89	35	14	21	23	30	29
43.48	-450	-8.48	278	5.25	22	15	7	30	26	23
45.10	-435	-10.14	329	7.67	25	13	12	27	28	32
54.89	-1000	-19.96	314	6.27	33	14	19	39	37	36

Day of Cycle (start of 7-d study)	Average of 3 men cycles	VO2 max (ml/kg/min)	VO2 max (L/min)	Heart Rate max (bpm)	VT (ml/kg/min)	VT (L/min)	VT (% of VO2 max)	90% of VT (L/min) (from VO2 max)	90% of VT (% VO2 max)
3	36.7	48.3	2.270	192	33.6	1.580	69.6	1.422	62.6
4	27.5	51.0	3.124	191	32.3	1.985	63.5	1.787	57.2
3	28.3	47.2	2.899	182	27.5	1.693	58.4	1.524	52.6
5	29.3	49.1	2.855		23.7	1.382	48.4	1.244	43.6
6	28.0	46.6	2.776	190	28.2	1.677	60.4	1.509	54.4
2	29.0	49.3	2.970	178	26.9	1.620	54.5	1.458	49.1
6	24.3	52.7	3.087	177	38.9	2.285	74.0	1.800	58.3
6	25.3	46.8	3.032	186	30.5	1.972	65.0	1.775	58.5
2	30.7	48.4	2.259	185	32.2	1.505	66.6	1.355	60.0
5	28.0	45.5	3.880	180	28.3	2.414	62.2	2.170	55.9
6	26.0	52.4	3.228	168	27.8	1.715	53.1	1.544	47.8
5	32.3	48.3	2.594	186	20.4	1.094	42.2	0.985	38.0
3	25.0	47.3	3.381	184	32.2	1.923	56.9	1.730	51.2
4	29.0	55.4	3.350	195	42.4	2.560	76.4	2.304	68.8
7	30.3	46.2	2.548	184	28.3	1.556	61.1	1.400	54.9
2	30.3	48.0	3.482	162	26.1	1.889	54.3	1.700	48.8
4	31.0	44.8	2.479	190	22.4	1.294	52.2	1.165	47.0
2	31.0	47.2	2.717	200	32.4	1.926	70.9	1.733	63.8
2	33.7	45.8	3.709	200	29.3	2.376	64.1	2.138	57.6
2	26.0	50.8	3.195	189	31.5	1.980	62.0	1.785	55.9
2	26.3	45.0	2.618	192	23.6	1.373	52.4	1.236	47.2
3	30.3	47.2	3.037	188	33.9	2.178	71.7	1.960	64.5
7	29.3	45.7	2.855	175	38.3	2.397	84.0	2.157	75.6
5	27.3	45.9	2.947	206	26.7	1.718	58.3	1.546	52.5
6	26.0	47.2	2.839	184	25.3	1.523	53.6	1.371	48.3
2	27.3	47.2	2.980	180	25.9	1.635	54.9	1.472	49.4
3	26.3	47.3	3.042	190	25.8	1.662	54.6	1.496	49.2
2	29.0	45.0	2.369	198	22.3	1.273	53.7	1.273	54.0
3	37.3	51.2	3.043	176	35.7	2.120	69.7	1.908	62.7

actual 4-d exercise (L/min)	actual 4-d exercise (% of VO2 max)	actual 4-d Heart Rates (bpm)	actual 4-d exercise (% HR max)	actual 4-d RER	actual 4-d exercise duration (min/day)	Ht (cm)	Wt (kg)	Percent Fat (%)
1.29	56.8	162	84.4	0.91	168	151.0	46.9	22.17
1.77	56.7	137	71.6	0.94	164	175.0	59.4	17.53
1.53	52.8	133	73.2	0.93	175	157.0	60.0	25.78
1.39	48.7	112	ERR	0.94	201	171.0	59.8	22.48
1.53	55.1	130	68.7	0.93	204	170.0	60.0	14.30
1.47	49.5	126	71.0	0.93	206	165.0	60.6	17.51
1.81	58.6	126	71.3	0.92	162	168.6	57.8	15.80
1.80	59.4	147	79.2	0.90	163	170.5	62.5	22.71
1.39	61.5	148	80.0	0.90	182	161.0	46.3	11.03
1.87	48.2	127	70.8	0.88	181	172.5	84.5	33.00
1.55	48.0	112	66.9	0.92	201	165.0	60.2	13.94
1.01	38.9	126	68.0	0.88	258	155.0	54.3	22.36
1.74	51.5	131	71.1	0.89	189	172.0	70.9	23.15
2.15	64.2	152	78.1	0.92	144	170.0	60.0	14.99
1.42	55.7	133	72.1	0.90	193	162.0	54.5	17.19
1.69	48.5	110	67.8	0.88	200	169.0	73.0	22.35
1.23	49.6	143	75.2	0.92	218	159.5	55.4	21.14
1.72	63.3	163	81.6	0.88	152	154.0	58.1	24.20
2.12	57.2	158	79.2	0.88	176	180.2	83.2	24.64
1.80	56.3	125	66.0	0.89	155	165.5	61.6	25.14
1.38	52.7	133	69.4	0.88	214	163.0	59.6	17.75
1.95	64.2	156	82.8	0.89	167	170.5	63.6	14.01
1.89	66.2	139	79.3	0.90	159	170.5	62.9	19.89
1.56	52.9	140	67.8	0.86	180	174.0	62.8	26.30
1.36	47.9	128	69.5	0.86	190	165.0	60.0	27.81
1.49	50.0	119	65.9	0.88	200	170.5	63.4	22.75
1.50	49.3	126	66.5	0.88	213	172.5	64.4	17.65
1.30	54.9	147	74.5	0.88	203	165.0	52.0	17.48
1.91	62.8	133	75.6	0.89	158	177.0	59.0	15.06

Fat Mass (kg)	Fat Free Mass (kg)	Triceps (mm)	Biceps (mm)	Subscapular (mm)	Midaxillary (mm)	Iliac Crest (mm)	Abdominal (mm)	Front Thigh (mm)
10.40	36.50	12.3	6.1	12.7	14.6	10.1	15.6	25.4
10.41	48.99	10.4	5.0	9.3	10.3	10.5	19.4	22.7
15.47	44.53	20.1	8.3	8.7	10.2	13.0	16.0	24.0
13.44	46.36	8.8	3.4	9.9	10.3	4.9	9.4	16.1
8.58	51.42	12.1	4.6	8.3	6.2	5.5	8.9	24.5
10.61	49.99	11.6	6.6	10.9	11.5	17.8	18.9	25.6
9.13	48.67	13.2	3.4	7.2	5.1	4.4	6.6	21.8
14.20	48.30	13.3	8.0	10.5	11.2	10.9	12.7	29.1
5.10	41.19	7.2	2.8	5.2	5.3	3.5	5.0	23.6
28.02	56.88	19.2	8.5	17.4	12.6	25.7	18.6	34.2
8.39	51.81	10.3	4.5	9.1	8.2	8.9	9.4	12.4
12.14	42.16	11.7	4.3	11.0	9.4	6.4	10.6	29.1
16.42	54.48	14.9	9.3	12.5	8.4	9.7	12.9	28.8
8.99	51.01	7.5	3.5	6.9	4.1	4.6	6.2	21.3
9.37	45.13	14.2	5.8	10.1	7.4	6.6	11.2	30.2
16.32	56.68	16.9	4.9	15.3	11.8	8.0	15.4	21.2
11.70	43.69	16.1	6.4	12.9	12.4	14.9	17.6	27.5
14.06	44.04	19.4	13.0	25.4	22.6	21.7	23.4	28.0
20.50	62.70	16.1	8.3	15.6	17.7	14.1	19.3	30.5
15.49	46.11	14.0	5.2	8.4	9.6	9.0	11.4	24.2
10.58	49.02	11.1	4.3	9.8	9.6	9.6	15.6	21.9
8.91	54.69	10.7	4.4	9.0	8.6	7.3	9.8	18.3
12.51	50.39	12.6	4.3	7.5	7.8	7.4	9.4	25.0
16.52	46.28	20.4	11.5	16.8	22.9	19.4	22.8	28.4
16.69	43.31	16.5	6.4	12.6	9.1	12.5	14.8	26.8
14.43	48.97	14.9	6.5	8.7	10.4	10.5	20.7	23.6
11.37	53.03	9.9	4.3	9.9	10.0	10.3	14.4	15.3
9.09	42.91	8.6	3.3	12.6	6.9	9.8	16.0	19.0
8.89	50.11	8.1	2.5	6.9	5.9	5.9	10.4	20.0

Rear Thigh (mm)	Medial Calf (mm)	5 skinfolds (CPAFLA) (mm)	4 skinfolds (Durnin & Womersley) (mm)	6 skinfolds (Yuhasz) (mm)	Wt (kg) Day 1	Wt (kg) Day 2	Wt (kg) Day 3	Wt (kg) Day 4	Wt (kg) Day 5
25.5	7.6	48.8	41.2	101.6	48.0	47.4	47.8	47.3	46.0
20.0	6.8	42.0	35.2	92.3	60.2	60.8	60.2	59.5	59.7
29.6	22.4	72.5	50.1	111.4	60.9	61.0	60.3	60.6	60.6
17.2	5.4	32.4	27.0	66.3	59.8	60.0	60.0	59.5	59.6
33.2	11.3	41.8	30.5	92.5	60.0	59.8	60.0	59.7	60.3
18.5	12.3	59.2	46.9	103.3	61.0	61.0	61.7	60.7	60.2
27.3	13.9	42.1	28.2	80.5	58.9	58.7	60.0	58.2	57.6
29.7	18.1	60.8	42.7	106.2	63.6	63.7	63.3	62.7	62.5
27.3	12.6	31.3	18.7	71.8		47.5	46.7	46.2	46.2
37.2	18.8	89.6	70.8	152.3	85.6	85.5	85.6	84.2	84.3
23.3	8.8	41.6	32.8	73.4	60.8	60.6	61.5	60.2	60.0
27.3	14.2	47.6	33.4	96.1	55.2	54.5	53.6	53.9	53.9
31.8	15.6	62.0	46.4	110.6	71.3	71.7	71.5	70.9	70.7
17.3	7.0	29.5	22.5	63.8	61.0	60.0	59.5	59.5	59.3
24.2	9.2	45.9	36.7	96.5	54.9	55.8	55.5	55.0	54.6
26.4	14.2	59.3	45.1	103.2	73.5	73.0	73.5	71.6	72.3
32.4	14.8	65.1	50.3	121.4	55.8	57.0	55.6	55.6	54.4
29.2	16.1	95.6	79.5	147.1	58.2	58.8	58.2	57.2	56.8
33.9	20.0	74.1	54.1	129.5	83.7	83.0	83.6	80.8	79.6
21.9	13.2	49.8	36.6	88.9	62.8	62.1	61.7	60.3	60.5
28.5	15.1	49.9	34.8	96.5	60.7	61.2	61.1	60.0	69.5
28.1	8.3	39.7	31.4	83.2	65.0	64.5	64.5	63.6	62.6
32.5	10.5	42.3	31.8	94.4		63.3	63.8	63.5	62.7
21.8	13.4	81.5	68.1	129.6	64.2	63.7	63.9	63.3	62.3
32.4	9.6	57.6	48.0	115.6	60.6	60.6	61.1	60.0	59.6
32.7	15.1	55.7	40.6	111.1	65.0	65.3	64.5	63.5	63.0
26.4	8.0	42.4	34.4	86.2	65.1	65.5	65.6	63.7	63.3
24.4	8.0	42.3	34.3	90.4	52.6	52.7	53.0	52.7	52.1
20.2	7.2	30.6	23.4	71.5	59.6	59.9	60.0	59.8	59.2

Wt (kg) Day 6	Wt (kg) Day 7	Wt (kg) average (Days 1,2,3)	Wt (kg) diff (Days 1,2,3 - 7)	Wt (kg) % diff (Days 1,2,3 - 7)	diff between 4-d & 7-d (kcal/day)	diff between 4-d & 7-d (kcal/FFM/day)	7-d diet (kcal/day)	7-d diet (kcal/FFM/day)
46.1	46.1	47.73	-1.63	-3.42	571	15.63	1809	49.57
59.7	60.5	60.40	0.10	0.17	1018	20.77	2427	49.55
60.7	60.6	60.73	-0.13	-0.22	1270	28.54	1860	41.76
59.9	59.0	59.93	-0.93	-1.56	45	0.97	3190	68.81
60.0	60.1	59.93	0.17	0.28	603	11.73	2987	58.10
60.5	60.7	61.23	-0.53	-0.87	1205	24.15	1465	29.30
58.5	58.7	59.20	-0.50	-0.84	2	0.04	2668	54.82
62.7	63.3	63.53	-0.23	-0.37	1027	21.27	1643	34.01
47.1	45.8	47.10	-1.30	-2.76	25	0.60	2225	54.03
84.2	84.4	85.57	-1.17	-1.36	1127	19.80	2003	35.22
60.5	60.2	60.97	-0.77	-1.26	920	17.75	1960	37.84
54.2	54.1	54.43	-0.33	-0.61	-639	-15.13	2724	64.60
70.0	71.3	71.50	-0.20	-0.28	910	16.70	1760	32.30
59.3	60.0	60.17	-0.17	-0.28	-384	-7.52	2909	57.02
54.4	54.6	55.40	-0.80	-1.44	-838	-18.58	3048	67.53
72.0	71.3	73.33	-2.03	-2.77	491	8.66	2284	40.29
55.5	55.0	56.13	-1.13	-2.02	449	10.27	1721	39.39
57.0	56.5	58.40	-1.90	-3.25	-141	-3.20	2016	45.77
78.9	78.7	83.43	-4.73	-5.67	208	3.32	2317	36.95
59.5	59.2	62.20	-3.00	-4.82	-685	-14.85	2605	56.49
59.3	59.1	61.00	-1.90	-3.11	-188	-3.84	2188	44.64
62.5	61.8	64.67	-2.87	-4.43	-66	-1.21	2316	42.35
62.5	62.2	63.55	-1.35	-2.12	-414	-8.21	2479	49.19
62.0	61.8	63.93	-2.13	-3.34	-1266	-27.33	2891	62.46
58.8	59.6	60.77	-1.17	-1.92	-156	-3.60	1656	38.24
62.7	62.5	64.93	-2.43	-3.75	-709	-14.47	2419	49.39
62.8	62.5	65.40	-2.90	-4.43	-450	-8.48	2305	43.46
52.0	52.0	52.77	-0.77	-1.45	-435	-10.14	1935	45.09
58.7	58.3	59.83	-1.53	-2.56	-1000	-19.96	2750	54.89

CHO (g)	CHO (kcal/day)	CHO (g/FFM/day)	CHO (%)	Protein (g)	Protein (kcal/day)	Protein (g/FFM/day)	Protein (%)	Fat (g)	7-day Fat (kcal/day)	Fat (g/FFM/day)	Fat (%)
206.5	826	5.66	43.5	77.0	308	2.11	17.2	57.9	521	1.59	30.6
398.6	1595	8.14	66.1	95.7	383	1.95	15.7	58.0	522	1.18	21.1
345.6	1382	7.76	74.9	66.3	265	1.49	13.9	35.8	322	0.80	17.0
508.9	2036	10.98	66.0	120.6	483	2.60	15.5	41.8	376	0.90	12.4
392.9	1572	7.64	52.8	110.3	441	2.15	14.7	117.2	1054	2.28	35.2
203.3	813	4.07	55.8	56.1	224	1.12	15.3	41.9	377	0.84	25.7
444.2	1777	9.13	66.9	97.5	390	2.00	14.7	56.4	507	1.16	19.1
265.5	1062	5.50	67.1	54.8	219	1.13	12.6	41.6	374	0.86	21.2
326.1	1304	7.92	58.2	76.2	305	1.85	14.0	42.8	385	1.04	18.1
348.0	1392	6.12	70.1	72.9	292	1.28	14.7	38.4	346	0.67	16.7
344.6	1378	6.65	70.6	69.0	276	1.33	14.1	35.7	321	0.69	16.1
318.8	1275	7.56	47.6	120.1	480	2.85	17.8	99.8	898	2.37	32.4
305.1	1220	5.60	68.0	67.2	269	1.23	15.8	33.2	299	0.61	17.5
458.2	1833	8.98	62.8	123.3	493	2.42	17.1	72.5	652	1.42	22.7
454.2	1817	10.06	59.6	100.2	401	2.22	13.2	91.2	820	2.02	26.9
333.2	1333	5.88	58.7	80.2	321	1.41	13.7	68.7	619	1.21	26.9
269.3	1077	6.16	60.0	73.5	294	1.68	16.0	46.8	421	1.07	23.0
272.6	1090	6.19	54.7	77.3	309	1.75	15.1	70.2	632	1.59	31.0
380.7	1523	6.07	65.0	85.8	343	1.37	15.4	58.6	528	0.94	22.9
448.2	1793	9.72	68.9	97.3	389	2.11	14.9	51.5	463	1.12	17.8
319.7	1279	6.52	58.5	96.5	386	1.97	18.0	61.3	552	1.25	25.0
317.8	1271	5.81	55.1	89.0	356	1.63	15.4	73.8	664	1.35	27.9
391.0	1564	7.76	63.5	83.9	335	1.66	13.6	65.5	590	1.30	23.6
337.1	1348	7.28	48.0	111.5	446	2.41	15.3	97.4	877	2.11	31.4
258.5	1034	5.97	61.7	49.0	196	1.13	11.8	49.7	448	1.15	27.8
341.4	1366	6.97	56.8	71.0	284	1.45	11.6	83.6	752	1.71	31.2
372.3	1489	7.02	64.7	65.3	261	1.23	11.4	66.3	597	1.25	25.9
241.2	965	5.62	49.0	79.0	316	1.84	16.0	71.8	646	1.67	33.0
429.8	1719	8.58	61.0	93.8	375	1.87	13.9	78.1	703	1.56	25.4

Ensure CHO (%)	Ensure Fat (%)	Ensure Protein (%)	CHO diff between 4-d & 7-d (%)	Fat diff between 4-d & 7-d (%)	Protein diff between 4-d & 7-d (%)
55	30	15	11.5	-0.6	-17.2
55	30	15	-11.1	8.9	-0.7
55	30	15	-19.9	13.0	1.1
55	30	15	-11.0	17.6	-0.5
55	30	15	2.2	-5.2	0.3
55	30	15	-0.8	4.3	-15.3
55	30	15	-11.9	10.9	0.3
55	30	15	-12.1	8.8	2.4
55	30	15	-3.2	11.9	1.0
55	30	15	-15.1	13.3	0.3
55	30	15	-15.6	13.9	0.9
55	30	15	7.4	-2.4	-17.8
55	30	15	-13.0	12.5	-0.8
55	30	15	-7.8	7.3	-2.1
55	30	15	-4.6	3.1	1.8
55	30	15	-3.7	3.1	1.3
55	30	15	-5.0	7.0	-1.0
55	30	15	0.3	-1.0	-15.1
55	30	15	-10.0	7.1	-0.4
55	30	15	-13.9	12.2	0.1
55	30	15	-3.5	5.0	-3.0
55	30	15	-0.1	2.1	-0.4
55	30	15	-8.5	6.4	1.4
55	30	15	7.0	-1.4	-15.3
55	30	15	-6.7	2.2	3.2
55	30	15	-1.8	-1.2	3.4
55	30	15	-9.7	4.1	3.6
55	30	15	6.0	-3.0	-1.0
55	30	15	-6.0	4.6	1.1